An assessment of the contribution of terrestrial organic matter to total organic matter in sediments in Scottish sea lochs

Thesis

AN ASSESSMENT OF THE CONTRIBUTION OF TERRESTRIAL ORGANIC MATTER TO TOTAL ORGANIC MATTER IN SEDIMENTS IN SCOTTISH SEA LOCHS

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ABSTRACT

The biogeochemical cycling of carbon in Lochs Creran and Etive was investigated by using lignin as the biomarker for terrestrial organic matter. The oxygen uptake rate, percentage organic matter due to loss on ignition, Rp index, total carbon, total organic carbon (TOC), total nitrogen, C/N ratio, and phosphate content were used as the proxies to determine the biodegradability of the sediment organic matter.

Total lignin in Lochs Creran and Etive ranged from 0.03 to 0.55mg/g, and Λ (mg/100mg OC) ranged from 0.37 to 0.98; the syringyl/vanillyl and cinnamyl/vanillyl ratios for both lochs ranged from 0.16-1.61 and 0-1.47, respectively. It was found that non-woody angiosperm tissues predominated in the lochs. The concentrations of lignin and the proxies were constant at individual locations, indicating continual but intermittent input of terrestrial materials into the lochs. However, total lignin and these proxies decreased significantly from the head to the mouth and outside the lochs, indicating the importance of the Rivers Creran and Etive in contributing terrestrial materials into the lochs and the importance of terrestrial organic matter in fuelling the biogeochemical cycling of organic matter in the lochs. Overall the effect of the hydrodynamic and hydrological regimes of the lochs, fish and shellfish farms, and bioturbation on the sediment organic matter was investigated.

From the head to mouth of the lochs, in Creran, lignin contributed to 0.69% and 0.47% TOC at LC0 and LC6; in Etive, lignin contributed to 0.91% and 0.33% TOC at RE2 and Camas Nathais, respectively. The input of organic matter into Creran was 1.9×10^9 g/year. Of this, 1.2×10^9 g/year (63.16%) was labile fraction and 7.0×10^8 g/year (36.84%) consisted of refractory organic matter. There were overall 5.0×10^8 gC/year input of total carbon and 2.5×10^6 g/year input of lignin materials into Creran.

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Specially dedicated to

My parents,

For your unconditional support

For everything,

And ∞

My sister and my brother,

For always being there

For your encouragement and support

CHAPTER 1 INTRODUCTION

1.1 GENERAL INTRODUCTION

Lignin is a recalcitrant component found only in vascular plant tissues. Hence lignin is used as the biomarker for terrestrial organic matter, as it also degrades very slowly and is preserved in the environments for a longer period of time to warrant its detection in the marine environment (see also Section 1.4.1.3). Previous authors had used lignin to study the contribution of terrestrial organic matter from river sources and the distribution of these materials in the coastal and marine environments (see references in Section 1.5.1.2). Usually lignin concentrations were correlated with stable carbon isotopes ratios (see references in Section 1.5.2) to study the relative distribution of terrestrial versus marine organic matter. Bulk elemental analyses were also carried out (see references in Section 1.5.5) as these terrestrial materials also contribute to a substantial amount of total carbon and total organic carbon in the sediment. In this research, lignin analysis was carried out for sediments along transects of Loch Creran and Loch Etive, in order to determine the contribution of riverine organic matter to the lochs, and the distribution of these terrestrial materials along both lochs. Apart from the lignin studies by Readman *et al.* (1986) and Reeves and Preston (1989; 1991) in the Tamar Estuary, this is the only study to determine the effect of terrestrial materials on transition waters on the west coast of Scotland or in the United Kingdom.

Overnell *et al.* (1995) found that the oxygen uptake rates and percentage organic matter due to loss on ignition decreased from the head to the mouth of Lochs Linnhe, Etive, Fyne and Goil. They suggested that this reflected the degradation of riverine particulate organic carbon (POC), most of which was expected to have settled near the heads. In this work, the oxygen uptake rates were compared with lignin concentrations in order to study the effect of terrestrial materials on the biodegradability of the sediment organic matter. The effect of terrestrial materials on the

biodegradability of the sediment organic matter was also determined by using other proxies (see Sections 1.1.1 and 1.5.2-1.5.6).

Ocean margins are regions adjacent to the continents, including inland seas, estuaries, and continental shelves and slopes, where the major portion of continental detritus delivered to the oceans is deposited (Blanton, 1991; Martin and Windom, 1991). Although the ocean margins comprise less than 20% of the surface area (Walsh, 1991) and volume of the ocean respectively, they account for up to 30% of ocean production because of the fertilizing influence of nutrient inputs from rivers, upwelling* and buoyancy exchanges at the shelf edge (Mantoura *et al.*, 1991). Over 90% of riverine particulates and associated carbon, trace metals and pollutants are trapped in the deltaic and shelf regions of the ocean margins. The extent to which ocean margins can trap or export terrestrial and autochthonous phytoplankton carbon profoundly affect global carbon mass balances and their climatic consequences (Mantoura *et al.*, 1991). The vast majority of the organic matter in oceanic sediments is located in deltaic, shelf plus upper slope sediments, which contain 44% and 42%, respectively, of the total organic matter in marine deposits (Chester, 2000). Hence sea lochs are ideal locations to study the transport and distribution of terrestrial organic matter. A sea loch or fjord is a geomorphologically well-defined entity. It is an easily defined, almost closed water body showing less variability than the open coast or ocean. It is an ecosystem of manageable size, hence it is possible to measure or estimate the in- and outflux of organic matter across the boundaries of their ecosystem (Brattegard, 1980). These are also important sites for the degradation and mineralization of biomass (Parkes and Buckingham, 1986). The relatively pollution free Scottish sea lochs are ideal systems to study the dispersal of organic matter (Cronin and Tyler, 1980), although there have been a great rise in fish farming activities (Nickell *et al.*, 2003; Pereira *et al.*, 2004; also see Figure 4.16).

Organic material from adjacent forests are commonly observed as floating debris, however only a small quantity of carbon is detected in the outflow (Grace and Malhi, 2002). According to

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* **upwelling** upward movement of deeper water under the influence of divergence of water at the surface, usually caused by differences in Ekman transport (Baretta-Bekker *et al.*, 1998).

Richey *et al.* (2002) most of the carbon across the Amazonia was exported through CO_2 evasion to the atmosphere (470 TgC/yr), this was about 13 times more than export of total organic carbon (36 TgC/yr) or DIC (35 TgC/yr; Richey *et al.*, 1990). However, there is still some way to go before the carbon budget could be balanced (Grace and Malhi, 2002). Although the Amazon basin is a great contrast to the lochs studied, this is one example where the carbon budgets in the aquatic environment could not be balanced. In this work, attempts have also been made to investigate what happens to the terrestrial organic matter in the Lochs Creran and Etive. Besides being used to construct the carbon budgets for Loch Creran these experimental parameters are also used to determine the processes occurring in the sediments within the lochs.

1.1.1 Hypotheses, aims and specific objectives

Hypothesis

1. River Creran at the head of Loch Creran and River Etive at the head of Loch Etive play an important role in transporting terrestrial debris into the lochs, hence the lignin, organic matter and carbon contents decrease further down the lochs.

2. Terrestrial organic matter plays an important role in governing the biogeochemical cycling of organic matter in the lochs.

3. Differences in the hydrodynamic and hydrographical regimes between Loch Creran and Loch Etive result in differences in the quantity and quality of the organic matter.

Aims

The aims of this research are:

1. To determine the sources and distribution of terrestrial organic matter in the two Scottish sea lochs, Loch Creran and Loch Etive.

2. To determine the contribution of terrestrial organic matter to total organic carbon.
3. To determine the effect of these terrestrial materials on the biodegradability of the sediment organic matter.

By the end of this work, I hope to construct a distribution profile for all the experimental parameters (lignin concentrations, as well as the proxies to determine the biodegradability of the sediment organic matter) along both Lochs Creran and Etive. The importance of the contribution of terrestrial organic matter to the sediment organic matter is determined from the percentage contribution of lignin to total organic carbon contents of the sediments. Correlations among all these parameters will determine the ability of these proxies to determine the biodegradability of the sediment organic matter. The importance of the terrestrial materials in governing the fate of the sediment organic matter in the lochs is also determined.

Specific objectives

1. In order to study the lignin distribution along the lochs, sediment samples were collected from several locations along transects of Lochs Creran and Etive. Locations outside the lochs were also samples as reference sites. As lignin serves as biomarker for terrestrial materials, detection of lignin along transects of the lochs represents the distribution of terrestrial organic matter along the lochs.

2. In order to study the seasonal variation of the lignin content, sediment samples were collected from certain locations on a monthly basis.

3. In an attempt to quantify lignin from the freshwater input, water samples were also collected upriver from the mouth of the River Creran. Lignin analyses were carried out for the dissolved and particulate fractions of the water sample.

4. A sediment trap was deployed in order to determine the quality and quantity of the sedimenting particles.

5. These experiments were carried out to determine the biodegradability of the sediment organic matter: oxygen uptake rates, percentage organic matter due to loss on ignition, Rp values, total carbon, total organic carbon, total nitrogen, C/N ratios and phosphate content. These were also carried out on a monthly and rotationally basis. Hence the seasonal variations, and the transect distribution of these parameters were also determined.

Other objectives

1. The analyses carried out in this research were the lignin analysis and the experiments to determine the biodegradability of the sediment organic matter. Prior to the analyses, the validity of each method was confirmed as detailed in Chapter 2. This was followed by determination of the reproducibility of the analytical methods.

2. The vanillic acid to vanillin ratio, (Ad/Al)v, was used to determine the degradation stage of the lignin material, as higher (Ad/Al)v value indicates more highly degraded material.

3. The lignin parameters S/V and C/V ratios, as well as the lignin compositional plots, were used to determine the vegetation source as different lignin oxidation products were produced by different vegetation type. The type of vegetation in both lochs determined using this method is then confirmed by lignin analyses of some known plant samples. This was further confirmed by comparison with the aerial photographs of Loch Creran.

4. Contribution of terrestrial and marine organic matter to total organic matter was determined. Contribution of lignin to total carbon, total organic carbon and terrestrial organic matter was also determined.

5. The efficiency of each parameter to serve as the proxy to determine the biodegradability of the sediment organic matter was investigated. The effect of terrestrial organic matter

on the biodegradability of sediment organic matter was investigated using regression analyses between lignin and these proxies.

6. The source contribution of organic matter into the lochs was determined. There are a few fish farming activities located along both lochs. The effect of these farms, as well as the effect of bioturbation on the quality of the sediment organic matter was also determined. The effects due to differences in hydrological and hydrodynamic regimes of the lochs on the quality and quantity of the organic matter were also investigated.

   The lignin, total organic matter and total carbon fluxes and budgets in Loch Creran were determined based on results obtained from the above.

1.1.2 About this chapter

Section 1.2 provides introduction to the organic matter, the production (Section 1.2.1) and decomposition of organic matter (Section 1.2.2), and factors controlling the preservation of organic matter (Section 1.2.3). Section 1.3 gives introduction to sea lochs: a brief introduction to the definition (Section 1.3.1.1) and characteristic of sea lochs (Section 1.3.1.2), and finally the details of the two lochs studied, Loch Creran (Section 1.3.2.1) and Loch Etive (Section 1.3.2.2). As this research is based mainly on lignin and the preservation of lignin materials in sediments, Section 1.4 is attributed to lignin: lignin properties (Section 1.4.1) and lignin biodegradation (Section 1.4.2). Introduction to lignin-decaying organisms (Section 1.4.3) and the aerobic and anaerobic degradation of lignin materials (Section 1.4.4) are also given, followed by a summary on lignin biodegradation (Section 1.4.5). In Section 1.5, the various analytical methods used in this research are introduced. Each of this analytical method: lignin analysis, oxygen uptake rate, carbon isotope determination, loss on ignition, C, N and P analyses, is given a brief introduction to the method. These are followed by literature review of previous attempts in the respective methods.

1.2 ORGANIC MATTER

Organic matter is composed mainly of carbon, nitrogen, oxygen, phosphorus and sulphur, is the most important electron donor in the marine environments, and provides energy for most of the biological reactions (Libes, 1992). Dead organic particles are fragments of animals, plants, and microorganisms, and these are known as detritus (Wotton, 1994). Almost all the organic carbon* on Earth is created through photosynthesis, whether on land or in water. But on land the process leaves characteristic fingerprints, as many land plants synthesize certain compounds, such as lignin or tannin, which are absent in the marine phytoplankton, hence this carbon of terrestrial origin should be traceable after it has entered the oceans. Thus the detection of these biomarkers in the sea can reveal if the carbon had a terrestrial origin (Ludwig, 2001).

The $^{13}C/^{12}C$ ratios and the lignin oxidation products are examples of fingerprints successfully used to provide valuable information on the transformation of organic matter as it travels from terrestrial to inland water to ocean ecosystems (Ward *et al.*, 1994). In this research, lignin was used as the biomarker to determine the sources and distribution of terrestrial organic matter, and the transport and fate of the terrestrial organic matter along the two lochs. Stable carbon isotope was used to determine the relative abundances between the terrestrial and marine organic matter.

1.2.1 Production of organic matter

The ultimate source of organic detritus is biological production in surface waters of the oceans and on land (Suess, 1980). Organic matter in the sea is produced by photosynthetic fixation of CO_2 and phytoplankton is the most abundant marine primary producer and the dominant source of organic matter in the ocean (Pomeroy, 1974; Finenko, 1978; Meyers, 1997; Chester, 2000; Bashkin, 2002). The average atomic ratio of the C:N:P in marine phytoplankton is 106:16:1.

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* **organic carbon** all chemical compounds with the reduced C atom as major constituent; molecules always contain H, O, as well as sometimes N, P, S, and also trace amounts of other elements; virtually all are formed during photosynthesis (Baretta-Bekker *et al.*, 1998).

This is known as the "Redfield-Richards Ratio" and the process of photosynthesis is represented as shown below (Libes, 1992).

$$106CO_2 + 122H_2O + 16HNO_3 + H_3PO_4 \longrightarrow \underset{\text{(organic matter)}}{(CH_2O)_{106}(NH_3)_{16}(H_3PO_4)} + 138O_2$$

On land, plants are the primary producer of organic matter (Campbell *et al.*, 1999). Lignin is a substance in plants used in this research. Figure 1.1 shows a simplified diagram of the production of lignin and its relation with the environments (Loh *et al.*, 2002).

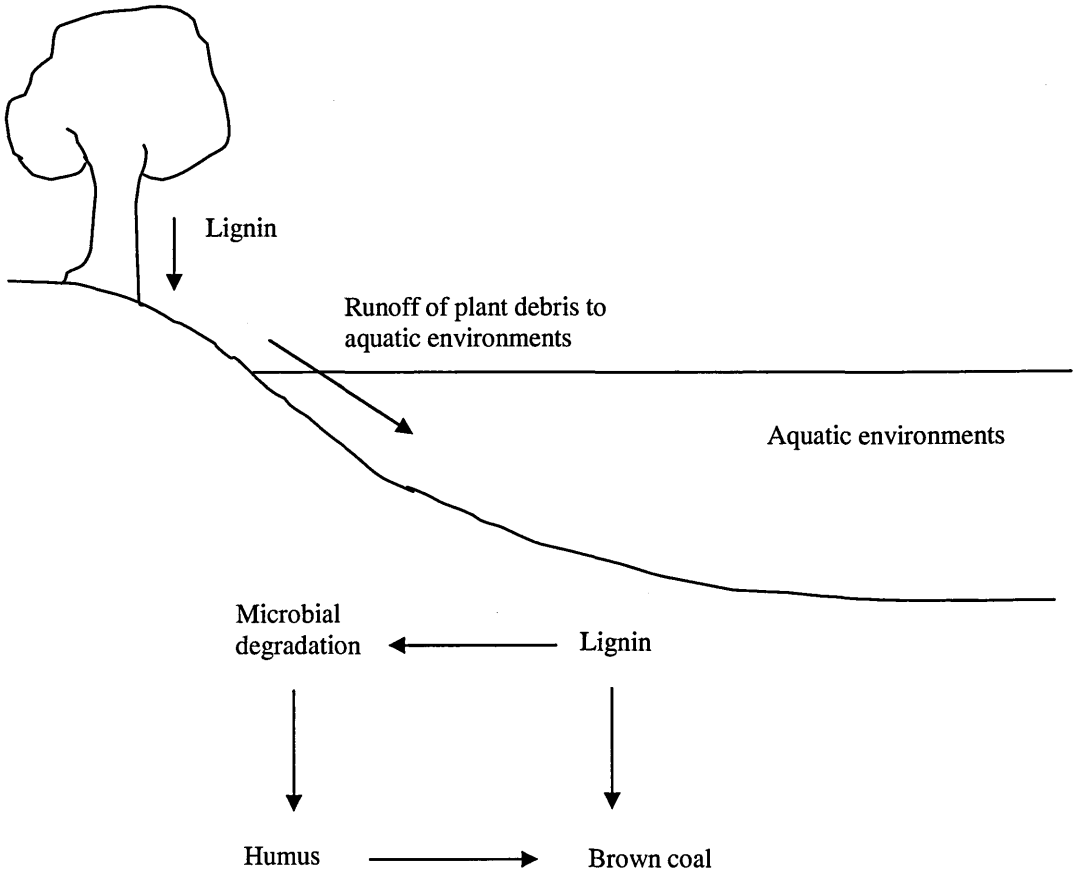

Figure 1.1 The lignin cycle (adapted from Christman and Oglesby, 1971).

1.2.2 Decomposition of organic matter

Together with the production of organic matter due to photosynthesis and the transformation of organic matter, decomposition* of organic mater and nutrient regeneration (remineralization) play the important role in organic matter cycling in an ecosystem (Sorokin, 1978; Neher *et al.*, 2003). All three terms: decomposition, degradation and mineralization give similar meanings (see footnote in previous page), hence in future all three terms are used to describe the breaking down of organic matter.

Particulate organic matter (POM) is composed of materials including excreta, molts, aggregated dissolved organic matter (DOM), and the living and dead tissues such as lignin. The nonliving or detrital fraction is decomposed by the respiratory activities of bacteria, fungi, protozoa, and animals. Decomposition of POM returns the nitrogen, phosphorus and carbon to their soluble forms hence this process is also known as nutrient regeneration or remineralization (Libes, 1992).

Most of the organic carbon introduced onto the shelves is mineralised in the water column and sediments (De Haas *et al.*, 2002). The decomposition of the organic compounds during sedimentation between the water column and surface sediments could be due to decomposition of the more labile fraction of the organic compounds as the labile, energy-rich substrates are turned over very rapidly (Wetzel, 1984). Only a small amount sinks quickly enough to reach the seafloor with less than 1% of POM produced by primary production survives through sinking (Libes, 1992). In this work, materials collected from the sediment traps were used to study the input of particulate materials (Vangriesheim and Khripounoff, 1990), and to measure the downward flux of particulates (Wassmann *et al.*, 1991). The amount of materials collected in the traps also gave an estimate of the annual organic input to the bottom sediment (Davies, 1975).

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* **decompose** break down of organic matter into constituent elements by bacterial or fungal action; break down into simpler chemical compounds.
degradation breakdown of a molecule into atoms or smaller molecules.
mineralise to convert organic matter into mineral (Collins English Dictionary).

Decomposition of organic matter is a useful indicator of the ecosystem conditions (Neher *et al.*, 2003). Carbohydrates, lipids and proteins are preferentially decomposed into soluble organic compounds available for subsequent mineralization (Conover, 1978; Lehmann *et al.*, 2002; Girisha *et al.*, 2003; Kalbitz *et al.*, 2003). The progressive exhaustion of the easily mineralizable compounds leads to the relative accumulation of recalcitrant materials such as lignin (Coûteaux *et al.*, 1991). Fast-growing micro-organisms that degrade the labile compounds are active in the early stage of decomposition, and this activity is enhanced by the availability of nitrogen. During this phase lignin content usually increased. During the later stages of decomposition, lignin decomposes (Girisha *et al.*, 2003).

Microfungi and bacteria participate in the aerobic degradation of lignin (Haider and Trojanowski, 1980). The micro-organisms that play an important role in lignin degradation are the white-rot fungi (see Section 1.4.4). Anaerobic degradation of lignin is extremely slow (see Section 1.4.5.2) and lignin and humus finding their way into anaerobic environments are protected from microbial attack, and along with other biosynthesized organics, are the probable precursors of coal (Christman and Oglesby, 1971).

1.2.3 Preservation of organic matter

1.2.3.1 Rate of organic matter mineralization

In the oxic layer of surficial sediments, most of the metabolizable organic carbon is mineralised. Preservation of organic carbon in sediments is related to the bulk sedimentation rate (Müller and Suess, 1979). At high deposition rate, such as the continental margins, oxygen availability is not the primary control of organic matter preservation (Cowie and Hedges, 1992) as most carbon decomposes by the anaerobic pathway (Canfield, 1994). The mineralization rate is determined by the quality of organic matter (Kristensen and Blackburn, 1987; Henrichs, 1992; Canfield,

1994; Kristensen *et al.*, 1995; Aller and Aller, 1998; Kristensen and Holmer, 2001); the fresher more labile fraction degrades faster compared to the more refractory fraction.

In this work, the quality of organic matter is determined by the Rp index (from the loss on ignition experiment) and the C/N ratio, and the rate of organic matter degradation measured using the oxygen uptake rates.

1.2.3.2 The effect of oxygen

The effect of oxygen on the degradation rate of sedimentary organic matter is a function of the lability of the decomposing material. Fresh material was depleted with little difference in rates in the presence or absence of oxygen, whereas old material was decomposed significantly (up to 3.6 times) faster with oxygen than without oxygen (Hulthe *et al.*, 1998). This implies that bioturbation, by exposing old buried material to oxygen, may enhance integrated organic carbon oxidation in marine sediments, and indicating that the oxic-anoxic-oxic redox transitions (deposition under oxic conditions, burial under anoxia and re-exposure to oxygen) promoted degradation (Hulthe *et al.*, 1998). Many burrowing infaunal taxa have the ability to transfer sedimenting particles, captured in the interface, rapidly to deeper levels in the sediment (Pearson, 2001). Anoxic bottom water conditions and lack of bioturbation, however, are factors preserving organic matter in carbon-rich sedimentary rocks (Reeburgh, 1995). In this work the effect of oxygen in preserving the organic matter is discussed in relation with the effect of bioturbation on the quality of the sediment organic matter.

Depletion in oxygen will elevate the sulphate reduction process in the marine environment (Jørgensen, 1982; Holmer, 1999). The rate of sulfate reduction is also directly proportional to the concentration of metabolizable organic carbon (Westrich and Berner, 1984); organic debris rich in phosphorus and nitrogen support the more rapid sulfate reduction. Organic matter degradation at the sediment water interface along both lochs Creran and Etive however, were not due to sulfate reduction as there was oxygen was present throughout the year (this study; Edwards and

Trusdale, 1997; Overnell *et al.*, 2002: see Section 4.2.1.3). However, even if sulfate reduction occurs deeper the sediments, terrestrial organic matter such as lignin is not a nutritious for sulfate reducing bacteria (Lyons and Gaudette, 1979).

1.2.3.3 Stabilization of organic matter

Organic carbon concentrations in the upper sediment column decrease down core by mineralization and biological uptake (De Hass and Van Weering, 1997). Besides the quality of organic matter and the effect of oxygen, factors that also play the role in preservation of organic matter are the stabilization of organic matter by its incorporation into mineral pores that are too small to allow entry and effective functioning by the enzymes that hydrolyze organic matter to a size amenable to biological uptake (Mayer, 1994), and the possible sorption of organic matter to mineral surfaces of fine grained sediments, causing a part of the labile fraction of organic matter to be unavailable for oxidation by micro-organisms (Keil *et al.*, 1994). Further aerobic and anaerobic bacterial attack on pre-existing organic material transported to the sea leads to a further increase in the proportion of biologically resistant materials and these may be preserved in the sediment (Moore, 1969).

1.3 THE STUDY OF SEA LOCHS

1.3.1 Definition of sea lochs

Some definitions of sea lochs or fjords are given. According to Overnell *et al.* (1995b), sea lochs are bodies of water appearing as invaginations[+] of the sea. They are fjord systems on the west coast of Scotland which are flooded, glacially over deepened valleys which may have one or more basins separated from the sea by shallow sills (Overnell, 2002). Sea lochs or fjords are glacially scoured, relatively deep bodies of water common to western mid-high latitude

[+] **invagination** a part folded back upon itself (Collins English Dictionary).

coastlines. The sheltered water and high sediment accumulation rates combined with the low-energy environments of sea lochs can result in the preservation of a very high-resolution climate record (Howe *et al.*, 2001). Fjords are transition zones between the land and the open ocean, regions of strong physical and chemical gradients, where fresh and salt waters mix and react; they are usually long and narrow with length many times greater than width, and have one or more sills (Cameron and Pritchard, 1963; Matthews and Heimdal, 1980; Pickard and Stanton, 1980). The estuarine circulation is a summer phenomenon where an inflow of the denser saline water from the sea enters below the outflowing less dense freshwater (Fairbridge, 1980; Sælen, 1980; Lewis, 1997; Nedwell *et al.*, 1999). In our lochs, estuarine circulation is present even in winter when there is high rainfall.

1.3.2 Characteristic of sea lochs

The head of the loch is the inland end, where there is usually a major river bringing in freshwater. The mouth of the loch is the seaward end. Freshwater input is the most important factor affecting the water properties in a loch. Contributions to the freshwater consist of the minor factor from precipitation on the water surface, and the major factor from river runoff. River runoff could be due to direct rainfall into the river and loch, and stored runoff in the form of winter snow melts. Freshwater from river runoff flows seaward in the upper layer, entraining salt water from below and carrying it out of the loch. Wind stress acting on the surface, freshwater supply and tides are the main energy sources (Svendsen, 1986). The narrows of lochs restrain the outflow of brackish water, inducing a re-entrainment of old river water to the plume (McClimans, 1980). In silled fjords water may be physically restrained in basin enclosures resulting in oxygen depletion and the water becomes anoxic (Skei, 1988).

1.4 LIGNIN

Organic matter of terrestrial origin is derived largely from the vascular plants and comprises a large and continually renewed pool of reactive organic matter in the estuarine and coastal marine environments (Moran *et al.*, 1991a; Opsahl and Benner, 1995). Plants are also the largest contributors of fixed carbon in the biosphere and the largest part of dead organic matter (Cain, 1980).

1.4.1 Properties of lignin

1.4.1.1 General introduction of lignin

The chief structural element of plants is comprised of cellulose, hemicellulose and lignin (Kratzl, 1965; Christman and Oglesby, 1971; Goring, 1971; Frankland, 1974); the amount of lignin present may vary between 15% and 35% (Kratzl, 1965).

According to Sarkanen and Ludwig (1971), the word "lignin" is derived from the Latin word "lignum" meaning wood, and indeed, lignin forms an essential component of the woody stems of arborescent gymnosperms and angiosperms in which their amounts range from 15% to 36%. Lignin comprises 20 to 30% dry weight of wood and is the most abundant source of benzene nucleus on the planet (Cain, 1980). Lignins are not, however, restricted to arborescent* plants, but are found as integral cell wall constituents in all vascular plants including the herbaceous varieties (Higuchi, 1980). Their presence has been demonstrated in tissues associated with stems as well as in foliage and roots (Young and Frazer, 1987). Lignin is the most abundant naturally occurring source of aromatic compounds, the second most abundant renewable organic material after cellulose (Young and Frazer, 1987), and the most abundant in terms of energy content (Kirk *et al.*, 1980).

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* **arborescent** having the shape or characteristic of a tree (Collins English Dictionary).

1.4.1.2 Lignin structure

The structure of lignin is based on the molecular units of the phenylpropane type (Wardrop, 1971; Eriksson *et al.*, 1990), and these phenylpropanoid structural units give the plants their rigidity and bind the fibre cell walls together (Eriksson *et al.*, 1990). Lignin is a unique polymer since it is comprised of non-repeating units and it is a three-dimensional array joined by carbon-carbon and ether bonds. Aerobic ring fission is known to be mediated by dioxygenase enzymes, which insert oxygen atoms onto the structure; oxygen serves as a reactant as well as an electron acceptor. A model structure of lignin (Figure 1.2) is obtained from Libes (1992).

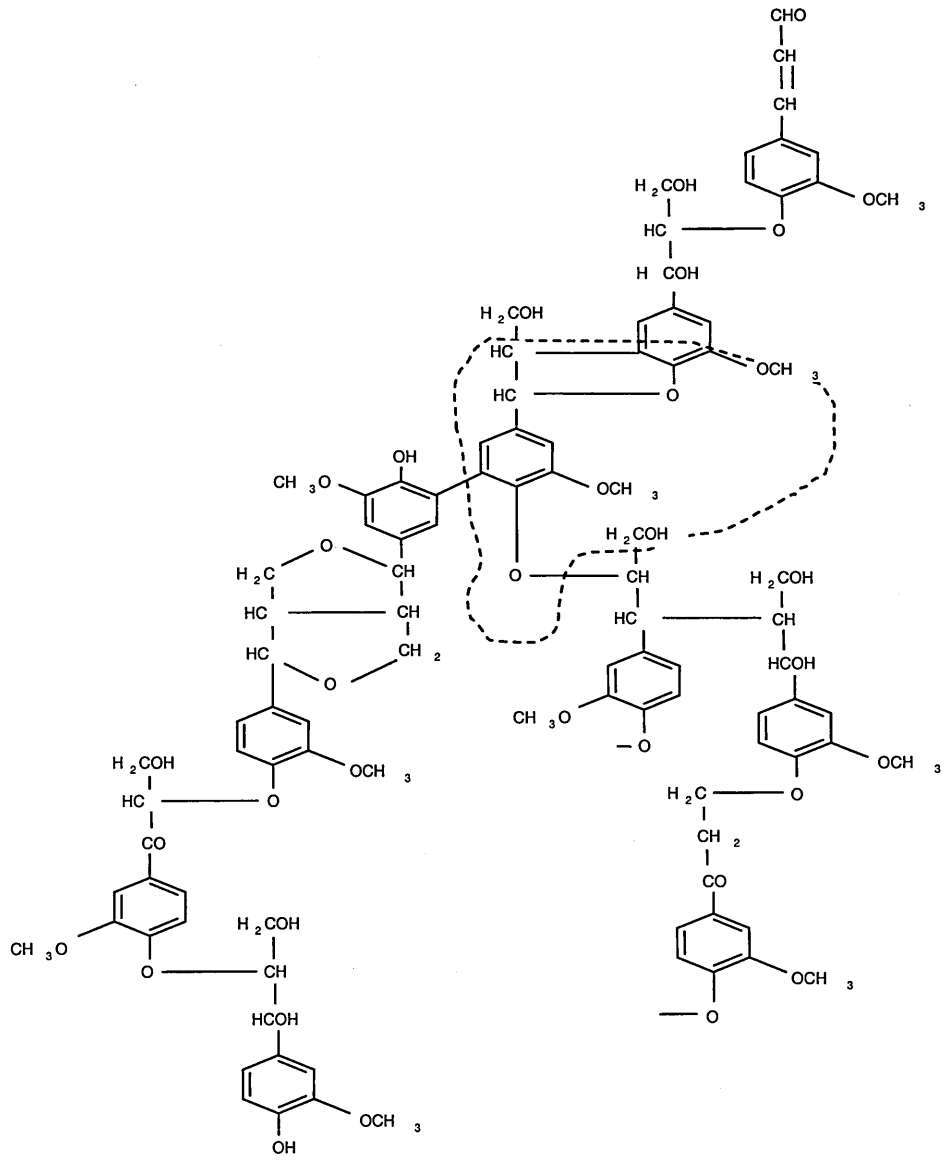

Figure 1.2 A model of lignin structure (Libes, 1992). The common structures of softwood lignin, with the major arylglycerol-β-aryl ether structure circled (Hammel, 1997).

1.4.1.3 Why is lignin used as biomarker?

Biomarkers such as lignin afford several advantages over measurements of bulk chemical properties such as elemental and stable isotope composition. The diversity and great number of different organic molecules in natural samples allow finer resolution of source contribution (Hedges and Prahl, 1993). For example, as lignin is found only in vascular plant tissues, the detection of lignin in sediments indicates contribution of terrestrial materials to the sediment organic matter. Secondly, biomarkers often occur in suites within which the relative abundances of the congeners* sometimes carry additional environmental or diagenetic information (Hedges and Prahl, 1993). For example, besides functioning as a vegetation source indicator, lignin parameters such as (Ad/Al)v ratios also indicate diagenesis+.

Lignin is a complex phenolic polymer constituting the major part of the cell walls of vascular plant tissues but absent from all other organisms (Hedges and Mann, 1979a; Hedges *et al.*, 1982), hence lignin oxidation products are useful for characterizing and quantifying vascular plant remains in freshwater systems (Hedges *et al.*, 1984).

Lignified tissues effectively resist attack by microorganisms by impeding penetration of destructive enzymes into the cell wall (Sarkanen and Ludwig, 1971), as lignin is an amorphus aromatic polymer, hence resistant to hydrolysis (Cain, 1980). Hence the stability of lignin to degradation enables the detection of this compound long after other more labile fractions has been degraded.

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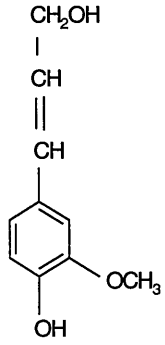
* **congener** member of a class, group, or other category, esp. any animal of a specific genus.

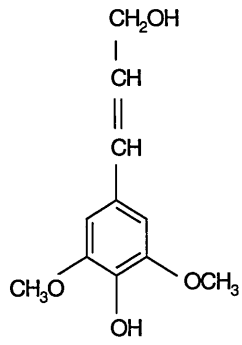
+ **diagenesis** the sum of the physical, chemical and biological changes that take place in sediments before they become consolidated into rocks, excluding weathering and metamorphic changes (Collins English Dictionary).

1.4.2 Lignin biodegradation

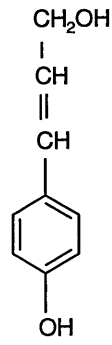
According to Sarkanen and Hergert (1971) the classification of lignins has so far not received extensive attention; most investigators have been satisfied with the broad division of better known lignins to (a) gymnosperm or softwood lignins, (b) angiosperm wood or hardwood lignins and (c) grass lignins. Gymnosperm lignin is a dehydrogenation polymer of coniferyl alcohol. Angiosperm lignin is a mixed dehydrogenation polymer of coniferyl and sinapyl alcohols. Grass lignin is composed of mixed dehydrogenation polymer of coniferyl, sinapyl and p-coumaryl alcohols (Higuchi, 1980):



Coniferyl alcohol



Sinapyl alcohol



p-Coumaryl alcohol

Chemical changes occurring during the initial stage of litter breakdown consist of decomposition of soluble carbohydrates, starches, pectin and soluble nitrogenous compounds, and the micro-organisms responsible for this initial attack comprise a variety of bacteria and fast-growing fungi (Jensen, 1974). The cellulose and hemicellulose degrade faster than lignin (McTiernan *et al.*, 2003). Thus, green pine needles contained a greater proportion of non-lignin fractions than freshly fallen needles, and decomposed at a much faster rate than the freshly fallen needles (Girisha *et al.*, 2003). The more resistant materials, especially lignin, tend to accumulate, and

with the disappearance of other more labile fractions soon form a major part of the organic residues (Hurst and Burges, 1967).

Lignin breakdown is generally a slow process both under natural conditions and in culture, and the yields of degradation products are usually low. The high molecular weight and low solubility of lignin precludes the possibility of direct assimilation and there are also the problems of fungal penetration and oxygen availability in large wood masses (Hurst and Burges, 1967).

Although lignin is relatively resistant to biological degradation, it does slowly and continuously, decomposes in soil to humus, water, and carbon dioxide following the death of the plant tissues (Hurst and Burges, 1967; Christman and Oglesby, 1971; Martin and Haider, 1980; Zeikus, 1980). Laccase and peroxidase are enzymes shown to attack lignin (Hall, 1980; Hammel, 1997). Generally the OCH_3 side chain, COOH carbons of phenolic units, amino acid, peptide and amino acid sugar unit carbons are degraded slightly faster than the benzene ring carbons. In time, most of the lignin fragments continue to decompose. The smaller lignin degradation fragments such as simple phenolic compounds, will become linked into the more complex soil humus polymers, and will become more stable and resistant to biodegradation. Decomposition of the more labile parts will release plant nutrient elements such as nitrogen and therefore important as a slow nutrient release fertilizer (Martin and Haider, 1980).

An example of the conversion of β-O-aryl lignin dimer by lignin peroxidase is given in Figure 1.3.

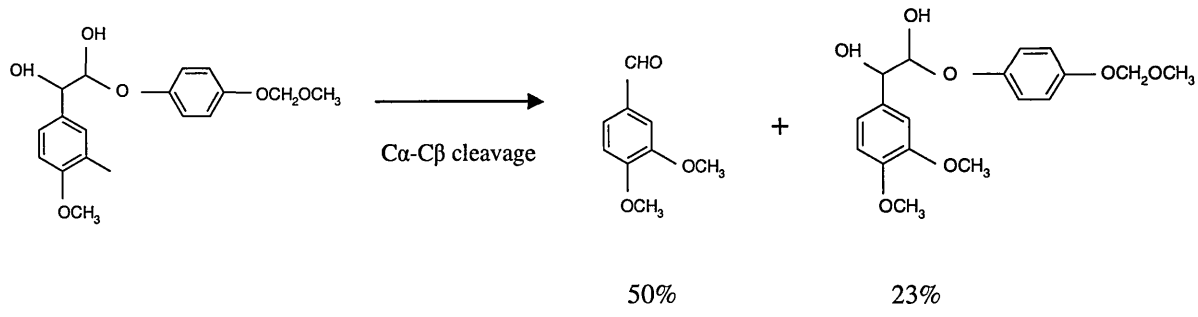


Figure 1.3 Conversion of β-O-aryl lignin dimer by lignin peroxidase (from Lewis and Yamamuro, 1990).

1.4.3 Lignin-decaying organisms

Micro-fungi and bacteria participate in lignin degradation in aerobic conditions (Haider and Trojanowski, 1980); fungi play the important role in lignin degradation (Crawford *et al.*, 1980), and other organisms such as bacteria and the actinomycetes to a lesser extent (Lewis and Yamamoto, 1990). Two main types of wood-decomposing fungi, usually referred to as brown rot and white rot, are distinguished (Grushnikov and Antropova, 1975).

Most of the fungal degradation of lignin occurs on land. In water fungi and bacteria degrade lignin but under anaerobic conditions lignin is not readily degradable (Edelkraut, 1996). It was found that the acid/aldehyde ratios of vascular plant debris often increased during subaerial degradation, but not further elevated within the aquatic environments (Hedges *et al.*, 1986; Ertel *et al.*, 1986; Hamilton and Hedges, 1988; Hedges *et al.*, 1988a). The observation that the acid/aldehyde values do not increase during lignin degradation in aquatic environments is suggestive that the aquatic bacteria degrade lignin side-chains and ring structures at similar rate (Benner *et al.*, 1991).

1.4.3.1 The white-rot fungi

The most well known lignin degrading micro-organism is the white-rot wood degrading fungi, some of which have been shown to destroy essentially all of the lignin in wood (Cain, 1980; Haider and Trojanowski, 1980; Coûteaux *et al.*, 1991). The chief are the white rot fungi belonging to the family Basidiomycetes (Christman and Oglesby, 1971; Eriksson *et al.*, 1990; Grushnikov and Antropova, 1975; Cain, 1980; Levy and Dickinson, 1981; Faison and Kirk, 1983; Hammel, 1997), several hundreds of which are from the family of Hymenomycetes (Chang *et al.*, 1980), and also certain wood-decomposing fungi which belong to the class of Ascomycetes (Grushnikov and Antropova, 1975).

The strategy of the white-rot fungi is to decompose the lignin in wood so that they can gain access to the cellulose and hemicellulose that are embedded in the lignin matrix (Hammel, 1997). Infection with white rot results in the decomposition of all the components of wood, and as decay proceeds the residue becomes paler in colour, compared with healthy wood. It was established that white rot fungi produce two main enzymes: laccase and tyrosinase. These enzymes catalyse the abstraction of electrons from phenolic substrates. In the presence of laccase and peroxidase one reducing equivalent is removed from the substrate, while in the presence of tyrosine two reducing equivalents are eliminated simultaneously (Grushnikov and Antropova, 1975). According to Filley *et al.* (2000), the white-rot fungal degradation is typically characterized by extensive propyl side chain oxidation and aromatic ring cleavage.

1.4.3.2 The brown-rot fungi

They do not degrade lignin extensively, although they modify it by demethylating it (Hammel, 1997). When wood is infected by brown rot, cellulose and other carbohydrate components are preferentially decomposed, while lignin remains relatively unchanged and the decaying wood acquires a brown colour (Grushnikov and Antropova, 1975). According to Haider and Trojanowski (1980), the brown rot fungi attack the methoxyl group more actively than the other carbons of the lignin skeleton. According to Goni and Thomas (2000), brown rot fungi are able to decay wood by selectively degrading its cellulose. Brown-rot fungi principally generate 3,4- and 4,5-dihydroxylated phenol groups (catechol derivatives) as a result of demethylation, and only to a small degree products of propyl side chain oxidation (Filley *et al.*, 2000).

1.4.3.3 The soft- rot fungi

The soft-rot fungi are Ascomycetes and Deuteromycetes that decay water-saturated (but not totally anaerobic) wood (Hammel, 1997). According to Nelson *et al.* (1995), soft-rot attack of both angiosperm woods resulted in the preferential removal of syringyl versus vanillyl phenols.

This trend is the observation that soft-rot fungi degrade gymnosperm to lesser extent than angiosperm lignin. Both the soft and white rot fungi were found to be able to release CO_2 from methoxyl or carboxyl groups, side chains of cinnamic acids or alcohols, or from the ring carbons (Grushnikov and Antropova, 1975).

1.4.4 Aerobic and anaerobic lignin degradation

1.4.4.1 Aerobic lignin biodegradation

Lignin biosynthesis and degradation are dependent on oxygen for different enzymes to be active. H_2O_2 used by peroxidase in the xylem tissues of trees and by the white-rot fungi, is produced from oxygen by oxidases. Lignin biodegradation is oxidative and several important oxidoreductase enzymes are dependent on oxygen or peroxidase for their activity. Thus, it is to be expected that lignin is not degraded anaerobically (Eriksson *et al.*, 1990).

Aerobic mineralization of lignocellulose is slow due to the impediment of cellulose decomposition by lignin, the inability of lignin to serve as a microbial energy source and increase recalcitrance of lignin during humification (Martin and Haider, 1980). Lignin has a compact structure that is insoluble in water and is difficult to wet and penetrate by micro-organisms. The inter-monomer linkages that account for the structural rigidity of lignin are a variety of C-C and C-ether bonds with the beta-aryl ether linkage being quantitatively the most significant, and these inter-monomer linkages are not directly hydrolysable (Zeikus, 1980).

The extracellular H_2O_2 and possibly superoxide ($O_2^{\cdot}$), play a role in lignin degradation (Forney *et al.*, 1982; Faison and Kirk, 1983).

1.4.4.2 Anaerobic lignin degradation

Anaerobic degradation of lignin is extremely slow due to several reasons. Lignin degradation requires substance such as glucose, the inability of lignin to serve as energy source and the increased recalcitrance of lignin during humification (Martin and Haider, 1980). Also, the absence of significant anaerobic decomposition of lignin in the biosphere is due to the mechanistic requirement of oxygen in biological catalysis to its degradation. Since lignin evolved in oxygen rich atmosphere, its synthesis requires oxygen, so it is logical that depolymerization of lignin also requires oxygen. Although lignin polymer is inert anaerobically, the aromatic alcohol precursors used in its synthesis are degradable (Zeikus *et al.*, 1982).

Hackett *et al.* (1977) found no 14C-labelled lignin biodegradation to labelled gaseous products under anaerobic conditions was observed. Odier and Monties (1983) also found no conversion of ^{14}C-labelled lignin to $^{14}CO_2$ or $^{14}CH_4$ was observed after 6 months of incubation at 30°C in anaerobic conditions with or without NO_3^-.

In the contrary, Healy and Young (1979) found that the range of 11 simple aromatic lignin derivatives is biodegradable to methane and carbon dioxide under strict anaerobic conditions: vanillin, vanillic acid, ferulic acid, cinnamic acid, benzoic acid, catechol, protocatechuic acid, phenol, p-hydroxybenzoic acid, syringic acid, and syringaldehyde. Benner *et al.* (1984) also found that lignin and lignified plant tissues are biodegradable in the absence of oxygen.

1.4.5 A summary on lignin biodegradation

Lignin biodegradation is a slow process (Section 1.4.2) even in aerobic environments (Section 1.4.4.1). Due to the complexity of its structure, under anoxic conditions lignin biodegradation is negligible. Some authors found that there is no lignin biodegradation occurring in anaerobic environments but others observed otherwise (Section 1.4.4.2); the reason could be because lignin

biodegradation in anaerobic environments occurs only for simple phenolic compounds (Healy and Young, 1979).

Although relatively resistant, lignin does slowly and continuously decompose (Hurst and Burges, 1967; Christman and Oglesby, 1971; Martin and Haider, 1980; Zeikus, 1980). As lignin is relatively resistant to biodegradation (Section 1.4.1.3), the more labile compounds are degraded first (Section 1.2.2); leaving the refractory fraction to accumulate in the organic residue relative to the more labile fraction (Hurst and Burges, 1967; Section 1.4.2).

In anaerobic environments, assuming that some of the more complex lignin material remain and accumulate with time, this results in detection of lignin materials thousand and million of years following. The lignin materials protected from microbial attack are the precursors of coal (Christman and Oglesby, 1971; Section 1.2.2). Due to its resistance to biodegradation and hence the ability to accumulate in sediment samples, lignin is used as the biomarker for terrestrial organic matter in sediments from Lochs Creran and Etive. Within the surface 10 cm sediment layers of Loch Creran lignin biodegradation still occurs, as observed from the (Ad/Al)v and Rp values.

1.5 THE ANALYTICAL METHODS

1.5.1 Analysis of Lignin

1.5.1.1 The history of lignin analysis

Early attempts at lignin analysis relied on acid hydrolysis and solvent extraction (Bader, 1956). Lignin was extracted from wood by removing all other organic matter and extracting lignin organic matter with solvent and followed by hydrolysis with HCl and H_2SO_4. Residual organic matter after H_2SO_4 treatment was known as lignin-humus (Pocklington and McGregor, 1973). Subsequently a different approach based on cupric oxide oxidation was developed (Hedges and Ertel, 1982). Cupric oxide oxidation requires less sample and solvent, and is much less time-consuming than previous methods compared to solvent extraction technique. Besides, according to Chang and Allan (1971), CuO has the appropriate oxidation potential for a suitable balance compared to oxides of mercury (II) and silver, as a weak oxidant will favour the oxidative coupling mechanism while a stronger oxidant will further oxidize the intermediate oxidation products. The historical development of the CuO oxidation method used for the analysis of lignin is shown in Table 1.1.

The refined method by Hedges and Ertel (1982) provides the basis for most laboratory lignin analyses today. Subsequent authors had used the method by Hedges and Ertel (1982): Hedges *et al.*, 1982; Ertel and Hedges, 1984; Hedges *et al.*, 1985; Ertel and Hedges, 1985; Wilson *et al.*, 1985; Prahl, 1985; Readman *et al.*, 1986; Requejo *et al.*, 1986; Hedges *et al.*, 1986; Ishiwatari and Uzaki, 1987; Hamilton and Hedges, 1988; Hedges *et al.*, 1988a and b; Reeves and Preston, 1989; Hedges and Weliky, 1989; Goni and Hedges, 1990a, b and c; Moran *et al.*, 1991a and b; Reeves and Preston, 1991; Cowie *et al.*, 1992; Goni and Hedges, 1992; Haddad *et al.*, 1992; Opsahl and Benner, 1993; Gough *et al.*, 1993; Goni *et al.*, 1993; Prahl *et al.*, 1994; Moran and Hodson, 1994; Goni and Hedges, 1995; Opsahl and Benner, 1995; Reeves, 1995; Edelkraut, 1996; Goni and Eglinton, 1996; Bergamaschi *et al.*, 1997; Bianchi *et al.*, 1997a and b; Bianchi

and Argyrou, 1997; Argyrou *et al.*, 1997; Louchouarn *et al.*, 1997; Goni *et al.*, 1998; Hu *et al.*, 1999, Keil *et al.*, 1998; Lobbes *et al.*, 2000; Onstad *et al.*, 2000; Mitra *et al.*, 2000; Miltner and Emeis, 2000; Dittmar and Lara, 2001; Ditmar *et al.*, 2001; Hernes *et al.*, 2001; Tareq *et al.*, 2004.

Some new developments of the method have also been published. Lobbes *et al.* (1999) had also used the CuO oxidation method developed by Hedges and Ertel (1982) for the oxidation and extraction of lignin material, but unlike most previous authors who used the gas-chromatography with flame ionisation detector (GC-FID), they successfully analysed the lignin-derived phenols using reversed-phase high-pressure liquid chromatography (RP-HPLC) with diode array detection.

Normally the samples, along with the CuO powder and NaOH solution, were heated in a muffle furnace for three hours (see Section 2.2.2 for further details). However, Goni and Montgomery (2000) successfully performed the CuO oxidation process using a microwave digestion system.

For this project, the CuO oxidation process was used; the analytical procedures are described in Section 2.2.2.

Table 1.1 Historical development of lignin analysis.

References (in chronological order)	Method	Sample and weight (g)	Reactants	Oxidation conditions	Internal standard
Bader (1956)	Acid hydrolysis and soxhlet extraction	50-100g sediment		Soxhlet extraction.	
Leo and Barghoorn (1970)	Nitrobenzene oxidation		15ml 8% NaOH, 1ml nitrobenzene	183°C, 2h	
Pocklington and MacGregor (1973)	Nitrobenzene oxidation	0.2g wood, 0.4g sediment	1ml nitrobenzene, 6ml 2N NaOH	170°C, 4h	
Hedges and Parker (1976)	CuO oxidation	100mg plant, 10g sediment	10g CuO, 100mg $Fe(NH_4)_2(SO_4)_2.6H_2O$, 150ml 8% NaOH.	170°C, 3h	(^{14}C) p-hydroxyacetophenone
Hedges and Ertel (1982)	CuO oxidation	25mg plant, 50mg humic acid, 0.1-2.0g soil/sediment	1g CuO, 25-100mg $Fe(NH_4)_2(SO_4)_2.6H_2O$, 7ml 8% NaOH	170°C, 3h	Ethyl vanillin
Hedges *et al.* (1982)	Hedges and Ertel (1982)	0.5-2.0g dry sediment		170°C	
Ertel *et al.* (1984)		20mg humic material		170°C, 3h	
Ertel and Hedges (1984)	Hedges and Ertel (1982)	0.5-1.0g sediment, 30mg humic acids			
Ertel and Hedges (1985)	Hedges and Ertel (1982), Ertel and Hedges (1984)			180°C	

Table 1.1 continued.

Reference	Method	Sample and weight (g)	Reactants	Oxidation conditions	Internal standard
Wilson *et al.* (1985)	Hedges and Ertel (1982)	2.5g sediment, 10-50mg plant	Ring-labelled (U-^{14}C) hydroxyacetophenone	170°C, 3h	0.5 μg μl^{-1} ethyl vanillin
Readman *et al.* (1986)	Hedges and Ertel (1982)	0.5g sediment	N_2 purged for 1h	170°C, 3h	
Requejo *et al.* (1986)	Hedges and Ertel (1982)	Used 1mm sieve	3h N_2 purging.		Ethyl vanillin
Reeves and Preston (1989)	Hedges and Ertel (1982)	0.5g sediment		170°C	100μg ethyl vanillin
Hedges and Weliky (1989)	Hedges and Ertel (1982)				
Goni and Hedges (1990a, b and c)	Hedges and Ertel (1982)	400mg sediment, 10mg conifer needles			
Moran *et al.* (1991a and b)	Hedges and Ertel (1982)	5-30mg humic substance		170°C, 3h	
Cowie *et al.* (1992)	Hamilton and Hedges (1988)				
Goni and Hedges (1992)				155°C, 3h	
Opsahl and Benner (1993)				155°C, 3h	
Gough *et al.* (1993)	Hedges and Ertel (1982), Requejo *et al.* (1986), Readman *et al.* (1986)	1g sediment sieved through 0.5mm sieve		170°C, 3h	10-100μg ethyl vanillin

Table 1.1 continued.

Reference	Method	Sample and weight (g)	Reactants	Oxidation conditions	Internal standard
Goni and Hedges (1995)	Hedges and Ertel (1982), Goni and Hedges (1992)			155°C, 3h	
Opsahl and Benner (1995)	Hedges and Ertel (1982), Goni and Hedges (1990b), Goni and Hedges (1992)	25mg sample		155°C, 3h	Ethyl vanillin (100µg)
Goni and Eglinton (1996)	Hedges and Ertel (1982), Goni and Hedges (1991)	5mg purified lignins, 20mg vascular plant tissues, 400mg sediment		155°C, 3h	
Keil *et al.* (1998)	Goni and Hedges (1990b)	300mg sediment		155°C, 3h	
Goni *et al.* (1998)	Hedges and Ertel (1982), Goni and Hedges (1992), Goni and Eglinton (1996)	400mg sediment		155°C, 3h	
Hu *et al.* (1999)	Hedges and Ertel (1982), Goni and Hedges (1990)	300mg sediment		155°C, 3h	
Lobbes *et al.* (2000)	Hedges and Ertel (1982)			150°C, 3h	
Onstad *et al.* (2000)	Hedges and Ertel (1982), Goni and Hedges (1992)	300mg dry sediment		155°C, 3h	
Mitra *et al.* (2000)	Hedges and Ertel (1982)				100µl ethyl vanillin
Miltner and Emeis (2000)	Hedges and Ertel (1982)		CuO, $(NH_4)_2Fe(SO_4)_2$, glucose, and 2M NaOH.		
Miltner and Emeis (2001)	Hedges and Ertel (1982)	500-2000mg sediment	2 mol/L NaOH, CuO, $(NH_4)_2Fe(SO_4)_2$.		

1.5.1.2 Lignin distributions in sediments

Some of the previous studies of lignin in sediment samples were carried out for marine sediments such as Washington continental shelf (Hedges and Mann, 1979b); the Gulf of Mexico and Washington slope (Hedges and van Green, 1982); Washington margin continental shelf and slope, the Cascadia Bay, and the Columbia River (Prahl *et al.*, 1997); Gulf of Mexico (Goni *et al.*, 1997, 1998); the Baltic Sea (Miltner and Emeis, 2001); and the Amazon basin late Pleistocene sediment (Kastner and Goni, 2003); from the river to coastal or marine sediments such as: Lake Washington Late Quaternary sediment (Hedges *et al.*, 1982); Columbia River (Hedges *et al.*, 1984); Beaufort shelf sediments (Goni *et al.*, 2000); the Mississippi River (Bianchi *et al.*, 2002); the Atchafalaya delta and bay region (Gordon and Goni, 2003); and sediments from estuaries such as the Tamar Estuary (Readman *et al.*, 1986) and the Narragansett Bay Estuary (Requejo *et al.*, 1986); and other locations such as Lake Biwa (Ishiwatari and Uzaki, 1987) and Rawa Dawau Lake (Tareq *et al.*, 2004). They had also used the S/V and C/V ratios to define the vegetation sources in the respective locations; so is this research (see Section 4.3). In this research, lignin analysis was carried out for sediments from two Scottish sea lochs (see Sections 1.3.2.1 and 1.3.2.2).

These authors had found that lignin content and the (Ad/Al)v value were constant at individual locations: Hedges and Mann (1979b), Hedges *et al.* (1982), Hedges and van Green (1982), Hedges *et al.* (1985), Readman *et al.* (1986), Requejo *et al.* (1986), Ishiwatari and Uzaki (1987), Hedges *et al.* (1988a), and Miltner and Emeis (2000, 2001). However, lignin content seems to decrease further from the freshwater input, and these authors had found so: Pocklington (1976), Hedges and Parker (1976), Hedges and Mann (1979b), Miltner and Emeis (2001) and Bianchi *et al.* (2002).

1.5.1.3 Lignin in the dissolved and particulate fractions

Rivers transport materials from land sources to the ocean (Duinker, 1980; Billen *et al.*, 1991; Liss *et al.*, 1991; Martin and Windom, 1991; Milliman, 1991; Paterson and Black, 1991) and are the dominant source of particulates for coastal waters (Moore, 1969; Ward *et al.*, 1994). Transport of organic matter from land to sea is a key link in the global carbon cycle because this is the most important pathway for the ultimate preservation of terrigenous production in modern environments (Hedges, 1992). According to Milliman (1991) rivers discharged about 35 x 10^3 km^3 of freshwater and 15 x 10^9 tons of sediment into the world oceans annually. The detection of lignin in the dissolved and particulate fractions of water sample collected from River Creran would indicate the transport of terrestrial materials from River Creran into Loch Creran. Also, fluxes of the particulate and dissolved materials discharged into the coastal zone by rivers have great influence on the bottom sediments and biodegradation processes (Cauwet and Mackenzie, 1993). This is because composition of organic matter affects the rate and extent of its degradation: the refractory lignin phenols and the ligneous part of the suspended organic matter are little degraded (Gadel *et al.*, 1993). Besides, vascular plant detritus degrades in terrestrial environments and coastal marshes, a significant fraction of the detritus is not directly mineralised but rather converted to dissolved decomposition products (Hodson and Moran, 1995).

The first and foremost lignin analysis for the dissolved and particulate materials was carried out by Meyers-Schulte and Hedges (1986). This was followed by the study of lignin in dissolved and particulate fractions: particulate material from the Amazon River (Hedges *et al.*, 1986), dissolved matter from the southeastern U. S. continental shelf and mangrove swamp (Moran *et al.*, 1991a,b), lignin in suspended and resuspended particles from the River Tamar Estuary (Reeves and Preston, 1991), dissolved organic matter from the Gulf of Mexico (Bianchi *et al.*, 1997b), particulate organic matter from the Baltic Sea (Bianchi *et al.*, 1997a), particulate organic carbon from Lake Pontchartrain Estuary (Bianchi and Argyrou., 1997; Argyrou *et al.*, 1997), dissolved organic matter from the Arctic Ocean (Opsahl *et al.*, 1999), suspended particulate material from

the Wight-Cherbourg transect (Ismaili *et al.*, 1999). Lobbes *et al.* (2000) collected water from Russian rivers entering the Arctic Ocean. They found that riverine organic matter was generally derived from plant and soil material and less from phytoplankton.

Procedures for sample collection and preparation prior to the analysis of lignin in the dissolved and particulate fractions of the water samples were given in Table 1.2. Different authors used different filter types and sizes, as well as different pre-combustion temperatures and times, and the drying procedures. The analysis of lignin is the same as the procedures used for the sediment samples. For our purpose the procedures followed for the extraction and analysis of the lignin materials in the dissolved and particulate fractions of the water sample collected from River Creran are described in Section 2.2.3.8.

Table 1.2 Methods for particulate and dissolved matter collection and processing.

Reference (year)	Water collection method					Analysis
	Filter type	Filter size	Filter precombustion	Water volume	Drying	
Eadie and Jeffrey (1973)	Glass fibre filter (Gelman A)	0.3μm	450°C for 4h			Inorganic carbon removed with 15ml 1N HCl (at 70°C for 16h). Samples ready for $\delta^{13}C$ analysis.
Cauwet and Mackenzie (1993)	Whatman GF/F		450°C, overnight		After filtration, filters dried at 50°C, 24h	For POC analysis.
Fichez *et al.* (1993)	Glass fibre filter (Whatman GF/C)		500°C, 4h		Filters dried at 50°C, 4h.	For $\delta^{13}C$ analysis.
Gadel *et al.* (1993)	Glass fibre filter (GF/F)		400°C		Samples freeze-dried	For CN analysis.
Coble (1996)	Nylon	0.45μm				
Argyrou *et al.* (1997)	Nucleopore	0.2μm				Lignin phenols.
Bianchi and Argyrou (1997)	Polycarbonate membrane filter and glass fibre filter	0.2μm and 0.7μm, respectively				For POC, PON and lignin phenols determination.

Table 1.2 continued.

Reference (year)	Water collection method					Analysis
	Filter type	Filter size	Filter precombustion	Water volume	Drying	
Ismaili *et al.* (1999)	Filter (Whatman GF/F)		450°C, 4h	20L water sample divided into four subsamples by filtration.	Filters rinsed with ultrapure water to eliminate salt and dried at 50°C for 12h.	For POC analysis.
Lobbes *et al.* (2000)	Glass fibre filter (Whatman GF/F)		500°C, 5h	100-200ml water was rotor-evaporated (for dissolved lignin determination).	Filtrate and filters stored frozen at – 30°C until analysis.	Lignin analysis for dissolved fraction of water sample.
Onstad *et al.* (2000)	Nucleopore or Millipore	0.4μm or 0.45μm, respectively		10-70L forced with compressed N_2 through 147mm filer, allowing collection ≈50mg to several grams particulate materials.	Resulting total suspended solids air-dried for about 24h.	For elemental, $\delta^{13}C$ and lignin analyses.
Dittmar *et al.* (2001)	Filter (Whatman GF/C)	1μm	450°C, 4h	250ml filtered through filters.		For particulate lignin and DOC analysis.

1.5.1.4 Other studies using lignin analysis

Other studies using lignin analyses are given below, among those are: the use tannin and cutin as substitutes for lignin as the biomarker for terrestrial organic matter, correlation of lignin concentration with carbohydrates and proteins, and the study of the effect of grain size and pollen on the lignin concentrations (Table 1.3).

Table 1.3 Other studies using lignin analysis.

Reference (year)	Researches
Ertel and Hedges (1985)	Using lignin of humic substances as indicator of terrestrial humic substances in coastal environments. Found that humic acids from fresh plants are more enriched in lignin components than carbohydrates.
Goni and Hedges (1990a)	CuO oxidation of purified apple (*Malus pumila*) cuticle and whole English Holly (*Ilex aquifolium*) leaves yields cutin acids in addition to lignin-derived phenols indicating that CuO oxidation can also be used for cutin analysis.
Goni and Hedges (1992)	Identified 32 lignin-derived phenolic dimers and 14 additional monomers.
Prahl *et al.* (1992)	Used diploptene as biomarker for terrestrial organic carbon.
Cowie *et al.* (1992)	Contribution of protein, polysaccharide and lignin to TOC in the sediment trap samples in order to obtain source information and to study the diagenetic processes of the sediment organic matter.
Gough *et al.* (1993)	Lignin and cutin were used to investigate the source and transport of plant materials.
Opsahl and Benner (1993)	Cellulose, hemicellulose, lignin and cutin.
Opsahl and Benner (1995)	Lignin and cutin
Bergamaschi *et al.* (1997)	Studied the effect of grain size and surface area on organic matter, lignin and carbohydrate concentrations.
Keil *et al.* (1998)	Studied the effect of grain size and surface area on amino acids, neutral sugars and lignin phenols.
Hu *et al.* (1999)	Studied the effect of pollen on lignin-phenol compositions.
Hernes *et al.* (2001)	Used tannin of lignin as a tracer for vascular plant tissues.
Hernes and Hedges (2004)	Tannin and lignin.

1.5.1.5 Lignin parameters

CuO oxidation is used to produce simple lignin-derived phenols to be analysed by gas chromatography. Upon CuO oxidation, lignin yields a suite of phenolic acids and aldehydes (Hedges *et al.*, 1982; Miltner and Emeis, 2000). There were altogether 11 lignin phenols used to determine the sources and diagenetic stage of the terrestrial organic matter: p-hydroxybenzaldehyde, p-hydroxyacetophenone, vanillin, acetovanillone, p-hydroxybenzoic acid, syringaldehyde, acetosyringone, vanillic acid, syringic acid, p-coumaric acid and ferulic acid. These can also be divided into the aldehyde, ketone and acid groups (Figure 1.4). These are detected by the gas chromatography in their trimethylsilated forms (these trimethylsilylated forms of lignin phenols are shown in **Appendix 1**).

Vegetation sources could then be determined as each of the 11 lignin phenols can be classified into three groups based on their structural monomer units (Higuchi, 1980): the syringyl, vanillyl and cinnamyl phenols. Syringyl phenols (S) are representative of woody and non-woody angiosperm tissues (Bianchi and Argyrou, 1997). These include syringaldehyde, acetosyringone and syringic acid. Vanillyl phenols (V), indicating the presence of gymnosperms tissues, include vanillin, acetovanillone and vanillic acid. The cinnamyl groups are found in non-woody angiosperms and gymnosperms (Bianchi and Argyrou, 1997), and these consist of p-coumaric acid and ferulic acid. Two useful parameters can be obtained from these lignin groups, the S/V and C/V ratios. The S/V ratio represents lignin from angiosperms versus gymnosperms and the C/V ratio represents woody versus non-woody tissues (Goni *et al.*, 1998).

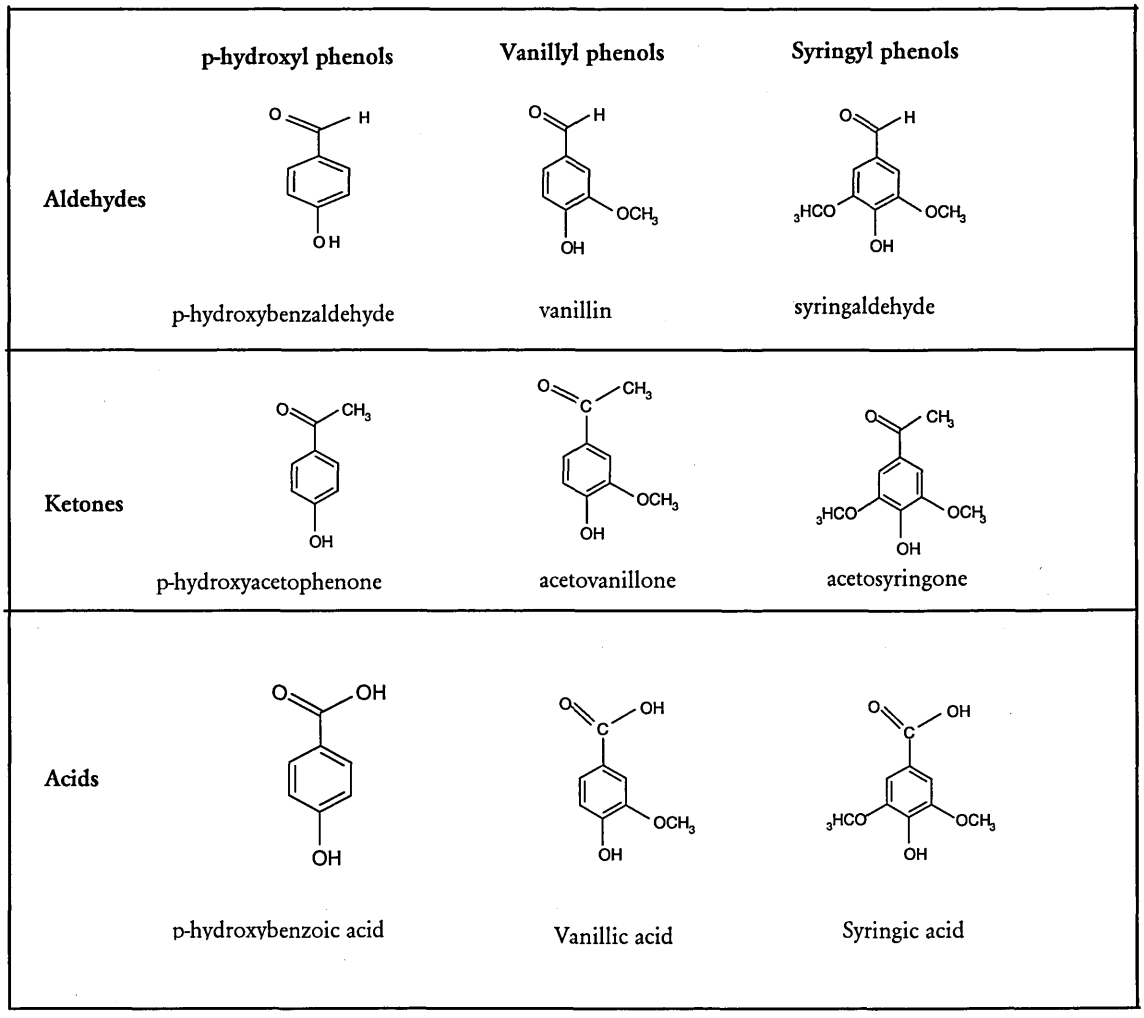


Figure 1.4 Structures of lignin in the aldehyde, ketone and acid groups (after Hedges and Parker, 1976). The two lignin phenols occurred only in acidic forms are the ferulic acid and p-coumaric acid. These two lignin phenols are not shown here. See also Appendix 1 for lignin phenols and their trimethylsilylated forms.

Hedges and Parker (1976) were the first authors to use λ (Greek small letter lambda) to describe the sum of the syringyl and vanillyl phenols normalized to 100mg [S+V (mg/100mgOC)], followed by Hedges and van Green (1982). Hedges and Mann (1979b) were the first authors who started the use of Λ (Greek capital letter lambda) to indicate the sum of the syringyl, vanillyl

and cinnamyl phenols per 100mg OC [S+V+C (mg/100mgOC)]. Since then Λ has become the most popularly used symbol for the definition of total lignin.

1.5.2 Determination of carbon isotope composition

$\delta^{13}C$ studies provide clues about the respective influence of the main source, the seasonal variation and nature of the organic matter sources that combine with the continental and marine end-members (Fichez *et al.*, 1993).

Carbon isotope fractionation is associated with photosynthesis. According to Smith and Epstein (1971), this fractionation results in lowering the $^{13}C/^{12}C$ ratio by about 20 per mille for land plants and 10 per mille for marine plants relative to atmospheric CO_2. Most land plants produce carbon that is more depleted in the heavy carbon isotope ^{13}C than carbon produced by marine phytoplankton, leading to higher isotopic ratios in the marine than in the terrestrial carbon (Ludwig, 2001). The $\delta^{13}C$ value of plants is also determined by the isotopic composition of their carbon source. For land plants this is atmospheric CO_2 [$\delta^{13}C$ (PDB) = -7‰]*, and for marine plants, carbon is assimilated as HCO_3^- [$\delta^{13}C$ (PDB) = ~0‰]. This causes most terrestrial C3$^{\phi}$ plants to have lower $\delta^{13}C$ (-26‰) than most marine phytoplankton which also use C3 metabolism. Also, plant metabolites such as proteins and carbohydrates are enriched in ^{13}C relative to cellulose, lipids and lignin. Since proteins and carbohydrates are more reactive, they are degraded first. Decomposition of these ^{13}C enriched compounds results in more depleted residual particulate materials (Libes, 1992).

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* **PDB** Pee Dee Belemnite (Bashkin, 2002).

$^{\phi}$ **C3** Most plants use the Calvin cycle for the initial steps that incorporate CO_2 into the organic material. They are called C3 plants, because the first stable intermediate formed by carbon fixation is 3-phosphoglycerate, a three-carbon compound (Campbell *et al.*, 1999).

Some authors who have used the stable isotopic ratio as an indicator of terrestrial organic matter (i.e., lignin in sediments) were: Hedges and Parker (1976), Hedges and Mann (1979b), Hedges and van Green (1982), Hedges *et al.* (1984), Wilson *et al.* (1985), Requejo *et al.* (1986), Cowie *et al.* (1992), Prahl *et al.* (1994), Goni and Eglinton (1996), Goni *et al.* (1997, 1998), Lobbes *et al.* (2000), Mitra *et al.* (2000), Onstad *et al.* (2000), Bianchi *et al.* (2002), Gordon and Goni (2003), and Tareq *et al.* (2004). Authors who have used stable isotopic ratio as an indicator of terrestrial organic matter, but not correlated with lignin were: Shultz and Calder (1976), Bird *et al.* (1995), Jasper and Gagosian (1989) and Quay *et al.* (1992). In this study the $\delta^{13}C$ values will be used to determine the relative abundance of terrestrial organic matter in terms of lignin versus marine organic matter.

These authors found that the $\delta^{13}C$ values increased further offshore from the freshwater input, indicating the presence of more abundant marine organic matter offshore relative to the terrestrial organic matter: Hedges and Parker (1976), Gearing *et al.* (1977), Tan and Strain (1979), Gearing *et al.* (1984), Fichez *et al.* (1993), Ruttenberg and Goni (1997), Cai *et al.* (1988), Yamamuro (2000) and Goni *et al.* (2000).

1.5.3 Oxygen uptake rates

According to Aure and Stigebrandt (1989), the rate of oxygen consumption in the basin water of a fjord (water trapped below sill depth) is a function of a number of factors such as the tidal and climatic conditions, the composition of the involved ecosystems, the topographic factors and local supplies of nutrients and freshwater.

Oxygen uptake by aerobic heterotrophic organisms depletes dissolved oxygen within the surface sediment layers. The oxygen uptake represents the amount of organic matter directly oxidised by oxygen (Overnell *et al.*, 1995b), hence the flux of organic matter is related to benthic oxygen uptake which is related to the ratio of carbon supply (Parsons *et al.*, 1977). Hence higher oxygen uptake rates represent higher sediment biodegradability due to higher abundance of the fresher

organic matter (Section 1.2.3), which provides the context for their use in the study being reported here.

Some past studies of the oxygen uptake rate were carried out by Parkes and Buckingham (1986), Nedwell *et al.* (1994), Overnell *et al.* (1995, a and b) and Nakamuro (2003). The procedures of the method followed in this study are obtained from Parkes and Buckingham (1986) and Overnell *et al.* (1995 a and b) and are shown in Table 1.4. The complete method used for the oxygen uptake rate measurements in this research is described in Section 2.2.4. The principle of the Winkler method is obtained from Hansen (1999; see reactions in Section 2.2.4). The Winkler method is an iodometric titration. Dissolved oxygen in seawater does not directly oxidize the iodide ion to iodine, so a multi-step oxidation is carried out using manganese as the transfer medium.

Table 1.4 Methods for oxygen uptake from intact sediment cores.

Reference	Oxygen uptake from intact sediment cores
Parkes and Buckingham (1986)	Sediment cores, with their top bung removed, were stored overnight in an all glass container. The container was aerated with water from the sampling site and *in situ* temperature to reduce disturbance as a result of sampling and subsequent transport. The cores in this container were covered with tops which incorporated an electronic magnetic stirrer each. Just before sealing (time zero), and at 3h and 6h after sealing, samples of the overlying water in the cores were taken for analysis of dissolved oxygen concentrations. Dissolved oxygen was determined using a Winkler amperometric back-titration in conjunction with an automatic titrator.
Overnell *et al.* (1995, a and b)	Oxygen uptake rates were determined from the decrease in dissolved oxygen in the overlying water in intact incubated sediment cores, as described by Parkes and Buckingham (1986). At time zero core tubes fitted with submersible stirrers enclosing a seawater space of about 12cm height above the sediment. Stirring speed was adjusted so that fine material at the sediment surface was not resuspended.

1.5.4 Loss on ignition

The loss on ignition (LOI) analysis was carried out in order to determine the percentages of labile, refractory and total organic matter. The relative abundance between the labile and refractory fractions of the organic matter is useful to determine the biodegradability of the sediment organic matter. The % labile organic matter is the percentage weight loss of the sediment sample after combustion at 250°C and the % refractory organic matter is the weight loss of the sediment sample after combustion at 500°C (Kristensen, 1990). Hence the % labile and % refractory organic matter serve only as operational definition for the relative abundance between the labile and refractory organic matter. Rp index, the ratio of the refractory to total organic matter, is also an important tool to measure diagenesis of organic matter, as a lower Rp value indicates the presence of more labile organic matter hence higher biodegradability of the sediment organic matter.

According to Mook and Hoskin (1982), high temperatures can cause weight loss in sediments through the loss of non-organic materials as well as organic materials. Kristensen and Andersen (1987) stated that the LOI peak at 150-300°C may not be entirely due to complete combustion of the organic matter, as evaporation of volatile low molecular compounds may be responsible for a certain unknown fraction. Christensen and Malmros (1982) showed that the conversion factors (LOI/C-content) increase with increasing depth, the main reason must be losses of inorganic material during ignition. Howard and Howard (1990) used a LOI and organic carbon content to estimate the soil organic matter, and applied the factor of 1.724. They observed that the use of LOI is complicated by the fact that ignition drives off not only organic matter but also water bound in clay minerals. Sutherland (1998) found that different soil types could have different conversion factors.

Previous experiments using the LOI analysis are given in Table 1.5. Although there seems to be quite a disparity in opinion concerning the use of this method to determine organic matter in sediments, LOI is used as a time saving, inexpensive and easy way to estimate the relative

amount of the labile and refractory fractions of the organic matter. The complete LOI analysis used in this research is described in Section 2.2.5.

Table 1.5 Methods for loss on ignition determination.

Reference	Pre-treatment	Method	Comments
Waksman and Hutchings (1935)	1.Dilute HCl and HF acids. 2.Complete combustion of organic matter. Calculation of total humus using an arbitary factor. 3.Oxidation of humus using strong oxidizing reagents. 4.Calculation of humus from total nitrogen.	Loss on ignition	1.Inaccurate: some components like uronic acid complexes destroyed. 2.Drawback: C content of humus depends on extent of their decomposition. 3.Method '4' is based on relation vary for different humus.
Hirota and Szyper (1975)		Separated organic and inorganic compounds by heating at 500°C.	
Froelich (1980)		1.Wet oxidation of OC. 2.OC = difference in TC before and after dry combustion at 450°C-600°C. 3.Sample leached with acid to eliminate $CaCO_3$, the residue analysed for TC.	1.Wet oxidation may be affected by redox interferences and inability to oxidize refractory organic materials completely. 2.OC not determined by difference on ignition (DOI) 3.Third method most accurate.
Mook and Hoskin (1982)		Organic material removed by digestion with 10% sodium hypochlorite at 100°C for 4h. Sediment ignited in a muffle furnace for 1, 2, 3, 4, 5, 6, 7 and 8h at 550°C. Sediment samples also ignited at 100, 200, 300, 400, 500, 600, 700 and 800°C for 4h.	Ignition at 550°C produced an average weight loss of 19.69±1.187%. Ignition at different temperatures showed significant weight losses between 200 and 300°C, and between 400 and 500°C. Results showed that high temperatures cause weight loss in sediments due to organic and non-organic materials, so LOI may give erroneous results.
Christensen and Malmros (1982)	Soil samples were oven-dried at 105°C for 24h and ground pass a 0.5mm mesh sieve.	LOI: igniting samples at 550°C for 4h. The conversion factor (LOI/C-content) as function of depth and spatial variability was determined.	Percentage loss due to inorganic materials is not constant for different C-contents. Authors recommended future studies to consider C-content instead of estimation of soil OM by LOI.

Table 1.5 continued.

Reference	Pre-treatment	Method	Comments
Gallardo *et al.* (1987)		OC was determined by ashing at 600°C or below 600°C.	
Kristensen and Andersen (1987)		1.TC in untreated dry sediments: CHN analysis. OC removed by combustion at 500°C for 16h. Residual IC was measured with the CHN analyser. OC = TC – IC. 2.IC was removed with 1N HCl: then analysed with CHN analyser.	Both methods gave similar results. Hence, DOI (difference on ignition) recommended as it is fast and without elaborate treatments to interfere the analytical equipment. The HCl method is time-consuming and the acid samples might destroy the interior of the CHN-analyzer.
Ben-Dor and Banin (1989)		1.Dichromate oxidation for % OC determination. 2.LOI: 400°C for 8h.	Good correlation between two methods, hence, LOI method was recommended due to its advantage of simplicity.
Howard and Howard (1990)		1.LOI: 550°C for 3h. 2.TC: dry combustion. 3.OC is used to estimate OM by a factor of 1.724.	This factor depends on soil type.
Bianchi and Argyrou (1997)		Percentage organic matter determined by weight loss after combustion at 450°C for 16h.	
Sutherland (1998)		1.OC: dry combustion analyser. 2.OM: LOI where samples heated at 450°C for 16h.	Advantages of the LOI method are: inexpensive, quick, requires no special training, and strong relationship between OM and OC determined with dry combustion.
Leong and Tanner (1999)		1.LOI: 0.5g sediment and ignited for 16h at 440°C, 550°C and 600°C. 2.OC was determined using a CHN analyser after removal of inorganic carbonates.	LOI is not reliable to determine OC in marine sediments, as loss of both organic and inorganic C may occur at the same temperature, and bound water is loss at elevated temperatures.

1.5.5 CN analysis

Besides the %TC and %TN contents of the sediment organic matter, the C/N ratio obtained from the elemental analysis can also be used to determine the degradation stage of the sediment organic matter. The C/N/P ratio also serves this purpose.

Nitrogen fluxes

Nitrogen is present in seawater as molecular nitrogen, inorganic salts such as nitrate (NO_3^-), nitrite (NO_2^-) and ammonia (NH_3), and organic compounds such as amino acids and urea, and particulate nitrogen. Nitrate, which originates mainly from soil leaching, terrestrial run-off (including that from fertilized soils) and waste inputs, is the most abundant stable inorganic species of nitrogen in well-oxygenated waters. The utilization of fixed nitrogen by phytoplankton takes place in the euphotic zone and some nitrogeneous compounds are released in a soluble form within this zone. The remainder are transported out by sinking particulates and a large fraction is released into the solution by remineralization of organic matter (Chester, 2000).

Above a critical rate of organic input to the sediment, an anaerobic layer develops in the sediment, and the contribution of nitrate to the total mineral nitrogen recycled to the water column decreases severely. Denitrification takes place in the anaerobic layer and reduces part of the nitrate produced by nitrification in the upper oxic layer. With a further increase of organic input, the upper oxic layer might become so reduced that nitrate production through nitrification decreases and limits denitrification in the lower layer (Billen *et al.*, 1991).

The analysis

In this research, the purpose of determining the carbon and nitrogen in the sediments is to determine the C/N ratio. Increase C/N ratio implies the presence of much non-living material of high C and decrease N content, for which lignin is a suitable candidate (Pocklington and MacGregor, 1973). According to Hedges *et al.* (1986) the general decrease in C/N with

increasing (Ad/Al)v indicate diagenesis. Hence the C/N ratio can be used to measure the degradation stage of the sediment organic matter.

Table 1.6 Methods for sediments CN analysis.

Reference	Sediments samples pre-treatment	CN analysis
Tanoue and Handa (1979)	0.6N HCl to remove carbonate	Organic carbon (OC) and total nitrogen (TN); CHN recorder
Pocklington and Leonard (1979)	Calibrated with acetanilide	
Hedges and Mann (1979b)	6N HCl to remove carbonates	Percentages by weight of OC; LECO carbon analyser
Hedges and van Green (1982)		OC and C/N; Carlo Erba 1106 elemental analyser; average reproducibilities (as a percent mean sample deviations) of ±1% and ±2%
Hedges *et al.* (1982)	2-20mg dry carbonate-free sediments	%OC and C/N, % mean deviations ±2% and ±3% respectively
Hedges and Stern (1984)	Treated sediments with HCl to remove inorganic carbon	
Mayer *et al.* (1985)	Sediments grounded and acidified with HCl	OC and N; analytical coefficient of variation was ±5%
Hamilton and Hedges (1988)	Sediments ground, sieved pass 350µm, treated with HCl to remove inorganic carbon	CN analysis, %OC and C/N reproducibilities as coefficient of variation were ±5% and ±16%
Kojima and Ohta (1989)	Sediment trap samples filtered through GF/C glass fibre filters, treated with 50ml 0.2N HCl and freeze-dried	OC and N; CHN analyser
Overnell and Young (1995)	For one sample, carbon was measured before and after heating in a muffle furnace furnace at 450°C for 24h. No carbon was found after heating, hence all carbon after heating was assumed to be organic	TC and N; LECO analyser Model CHN-900
Mayer (1994)	Ground and acidified to remove carbonate	
Hu *et al.* (1999)	Dry carbonate-free sediments used	Average reproducibilities were ±2% and ±3% for %OC and atomic C/N ratio
Onstad *et al.* (2000)	HCl treatment to remove carbonates in sediments	%OC and %TN; Carlo Erba Model 1100 or 1500 elemental analyser, Precision was ±2%
Bianchi *et al.* (2002)	Oven-dried at 50°C for 24h, 100mg sediment acidified with acid vapour, then dried at 50°C for 12h	C and N; elemental Analyzer (EA1108 FISONS Instruments)

Examples for the procedures followed prior to the CN analysis, especially the elimination of the carbonate fraction, and the CN analysis, are given in Table 1.6. The procedures followed in this research for the CN analysis, with and without the elimination of the carbonate content, are given in Section 2.2.6.

1.5.6 Phosphate analysis

Phosphorus fluxes

The global fluxes are dominated by the essentially one way flow of phosphorus carried in eroded materials and wastewater from the land to the oceans, where it is ultimately buried in oceans sediments (Bashkin, 2002). Phosphorus is present in river waters in dissolved and particulate forms. The dissolved phosphorus is mainly orthophosphate (principal species HPO_4^{3-}), together with dissolved organic phosphate and, in polluted systems, polyphosphate (Chester, 2000). In relatively shallow environments such as lakes, estuaries and continental shelves, sediments play an important role in the regeneration of phosphate (Fisher *et al.*, 1982). The removal of dissolved inorganic phosphate from solution by adsorption onto particles was suggested (Froelich, 1988), and the particulate inorganic phosphate is resupplied in the water column during resuspension (Edmond *et al.*, 1985; Berner and Rao, 1994; Balls, 1994). The reactions that release phosphate to the pore water are the release of adsorbed phosphate during reduction of ferric oxyhydroxides, organic matter decomposition and desorption of phosphate from surface sites on sediment particles (Krom and Benner, 1981). The surface adsorbed phosphate is released to the pore water to maintain the equilibrium concentration and replace the dissolve phosphate that escapes to the overlying water. Sedimentation and biological mixing transport adsorbed, sequestered, and organic phosphate downward into the reducing region of the sediment, and bioenhanced diffusion transports dissolved phosphate upward toward the sediment surface (Sundby *et al.*, 1992).

Phosphorus is even less abundant in biota than nitrogen. The use of organic carbon, nitrogen and phosphorus ratios (C:N:P) have been employed as source indicators of organic matter (Yamamuro, 2000). The methods used by previous authors for the phosphate analyses are given in Table 1.7. In this research, the procedures followed for the inorganic and organic phosphate analyses (see Section 2.2.8) were obtained from Strickland and Parsons (1972), Aspila *et al.* (1976) and Koroleff (1976).

Table 1.7 Methods for phosphate analysis.

Reference	Reagents used in phosphate analysis
Riley and Skirrow (1965)	- Phosphate in seawater: sample reacts with acidified molybdate reagent to yield phosphomolybdate complex, which is reduced to phosphomolybdate blue of which the extinction is measured. - TP: preliminary oxidation or hydrolysis of the organic phosphorus compounds. Orthophosphate formed determined by molybdenum blue.
Aspila *et al.* (1976)	TP is extracted from sediments with 1N HCl after ignition at 550°C or by digestion with sulphuric acid-potassium persulphate at 135°C in a sealed PTFE-lined Parr bomb. OP determined by difference in P content of the 1N HCl extract before and after ignition at 550°C.
Koroleff (1976)	- Mixed reagent I: Add 45ml molybdate solution to 200ml of the 9N sulphuric acid and then add 5ml tartrate solution. - Mixed reagent II: Add 45ml molybdate solution to 120ml of 9N sulphuric acid. Add 5ml tartrate solution and 70ml distilled water. - Ascorbic acid solution: Dissolve 7g ascorbic acid in 100ml distilled water. - Phosphate standard stock solution: Potassium dihydrogen phosphate KH_2PO_4 is dried in oven at 110C then in a desiccator. Exactly 136.1mg is dissolved in distilled water to which has been added 1ml 9N sulphuric acid. Finally dilute to 100ml
Van Veldhoven and Mannaerts (1987)	- Inorganic phosphate assay: To a 1ml sample, in plastic or acid-washed glass tubes, containing up to 20nmol Pi, 0.2ml reagent A was added. After 10min at room temperature 0.2ml reagent C was added. Absorbance at 610nm was determined 30min later. - Organic phosphate: After wet digestion of organic compounds, organic phosphate determined in the same nanomolar range. - Reagent A: 1.75% (w/v) ammonium heptamolybdate.$4H_2O$ in 6.3N H_2SO_4 (stable at room temperature) - Reagent C: 0.035% (w/v) malachite green, 0.35% (w/v) polyvinyl alcohol, PVA in water (dissolve PVA in water at 80°C, let cool, add dye, and bring to volume; stable at room temperature)
Liebezeit (1991)	About 50mg of sample was weighed into glass containers and extracted with doubly distilled water for 24h at ambient temperature with occasional shaking. A second set of samples was treated with 1N HCl for 14h at ambient temperature. A third set was combusted at 550°C for 1h prior to acid extraction. The first assay gives water extractable P (P_w), the second total inorganic phosphate (P_I), and the third total P (P_T). Organic P (P_O) is calculated as difference between P_T and P_I.
Schenau and de Lange (2001)	Used methods of Strickland and Parsons (1968).

TP = total phosphate, OP = organic phosphate

CHAPTER 2 MATERIALS AND METHODS

In this chapter, the information of the lochs are given in Section 2.1, Loch Creran in Section 2.1.1 and Loch Etive in Section 2.1.2, and the study areas in Section 2.1.3. Details on the analytical methods are given in Section 2.2. Section 2.2.1 details the sampling method and samples pre-treatment. The method for lignin analysis is given in Section 2.2.2, followed by the lignin analysis validation experiments in Section 2.2.3. The methods for other analyses are given in these sections: oxygen uptake from intact sediment core (Section 2.2.4), loss on ignition along with the method validation experiments (Section 2.2.5), CN analysis (Section 2.2.6), stable carbon isotopes (Section 2.2.7), phosphate analysis (Section 2.2.8) and the methods for data analyses (Section 2.2.9).

In this research the two lochs studied, Loch Creran and Loch Etive, were situated on the west coast of Scotland (Figure 2.1). Sediments were collected from various locations along transects of both lochs. The coordinates of these locations are given in the following sections.

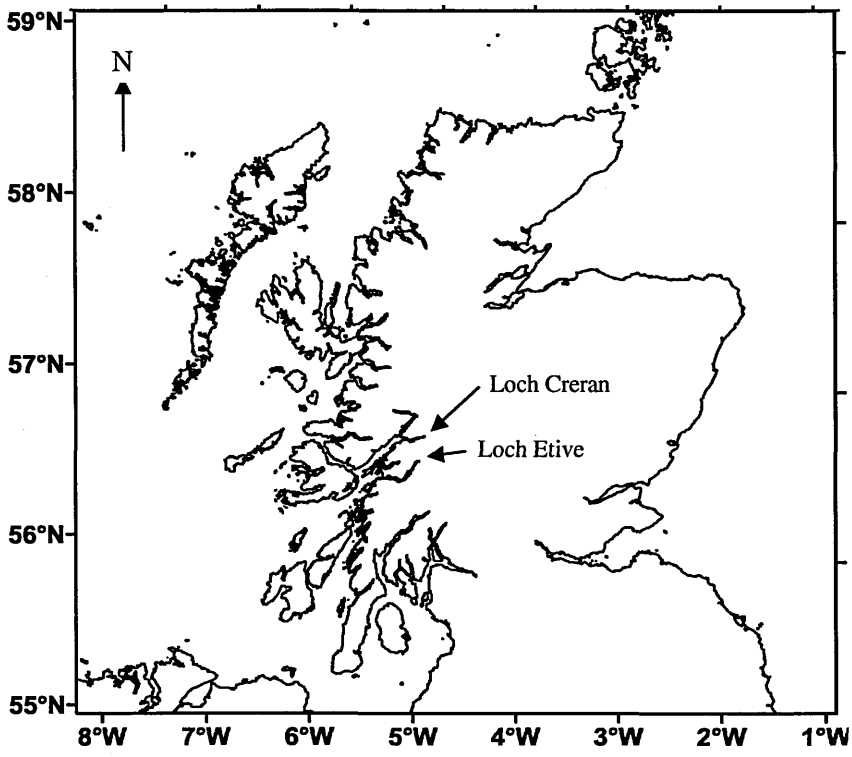

Figure 2.1 Map of Scotland showing Loch Creran and Loch Etive.

2.1 LOCHS CRERAN AND ETIVE

Loch Etive and Loch Creran are neighbouring sea lochs situated along the fjordic coastline of the west coast of Scotland. Both lochs show features in common of their origin as submerged, glaciated river valleys. Each loch is a double basin system interconnected, and linked to the outside sea areas, by shallow rocky sills (Gage, 1972). In each, the deep basins of the loch open to the sea by way of shallow, narrow sills, and each loch is divided into two or more basins by further shallow sills (Ansell, 1974).

There are several hydrographic contrasts between lochs Creran and Etive. The most important difference lies in the amount of freshwater runoff in relation to the total loch volume and its degree of tidal flushing. In Loch Etive, the large volumes of freshwater entering the loch effectively control a process of occasional ventilation of the deep basins which in the absence of

ventilation might otherwise become stagnant. In Loch Creran, on the other hand, runoff effects are only slight, and tidal flushing is sufficient to ensure ventilation of the bottom water. Loch Creran is shallower (max. depth 46m) and the entrance narrows are of roughly similar depth and dimensions. Also there is no modification of the tidal cycle in Loch Creran as occurs in Loch Etive. Exchange and mixing with outside seawater, generated by the tidal currents are therefore quite rapid (Gage, 1972).

2.1.1 Loch Creran

2.1.1.1 Hydrographic conditions

Loch Creran has only a small catchment and the seasonal hydrography closely follows the pattern outside. In the main basin, the amplitude and timing of the tidal cycle is not modified by the topography of the loch as it is in Loch Etive. Because of this and its generally shallower nature compared with Loch Etive, relatively stronger tidal currents affect the bottom.

Salinities near the loch bottom were comparable to those found outside, showing a variation of less than 1 for the bottom as a whole at any one time. At the surface there were indications of the effect of freshwater with the lowest salinity of 24.6 (Gage, 1972). Surface salinity fluctuations occur at times of heavy rainfall, and these reductions in salinity, because of the effect of turbulent mixing of surface and deeper waters in the area of the narrow sills, may lead to reduction in salinity throughout the water column although, in general, this effect is lower in Loch Etive (Ansell, 1974).

Slightly cooler water is usually found at the surface, associated with surface freshening from land runoff. The bottom temperatures within the loch are usually slightly higher than outside, indicating that considerable vertical mixing does occur, most probably in the shallower, more turbulent areas of the main basin. Oxygen concentration does not drop below 87% saturation (Gage, 1972).

2.1.1.2 Geography

Loch Creran possesses a shallow sill at the entrance to the main basin, which covers 11.49km^2. The other sill at Creagan, enclosing a small upper basin that has a surface area of 2.04km^2 (Gage, 1972). Loch Creran has a small catchment area of 164km^2 (Edwards and Sharples, 1986).

The following description of Loch Creran is taken from Edwards and Sharples (1986). Loch Creran is 12.8 km long and consists of four basins. The first basin, 27m at the deepest, is nearest the mouth and is separated from the Lynn of Lorn with a sill of approximately 4m deep. A sill of 6m deep separates this first basin from a second basin. The second basin, down to 49m, is the deepest of the four basins and is separated from the third basin by a 10m-sill. This third basin, 27m at the deepest, is, the widest of the four basins. The forth basin, 37m at the deepest, is the smallest basin and is separated from the previous basin with a 1m sill at Creagan narrows. The locations of the sills, as well as the depths of the sills and basins, are illustrated in Figure 2.2. River Creran at the head of the loch is the main freshwater source.

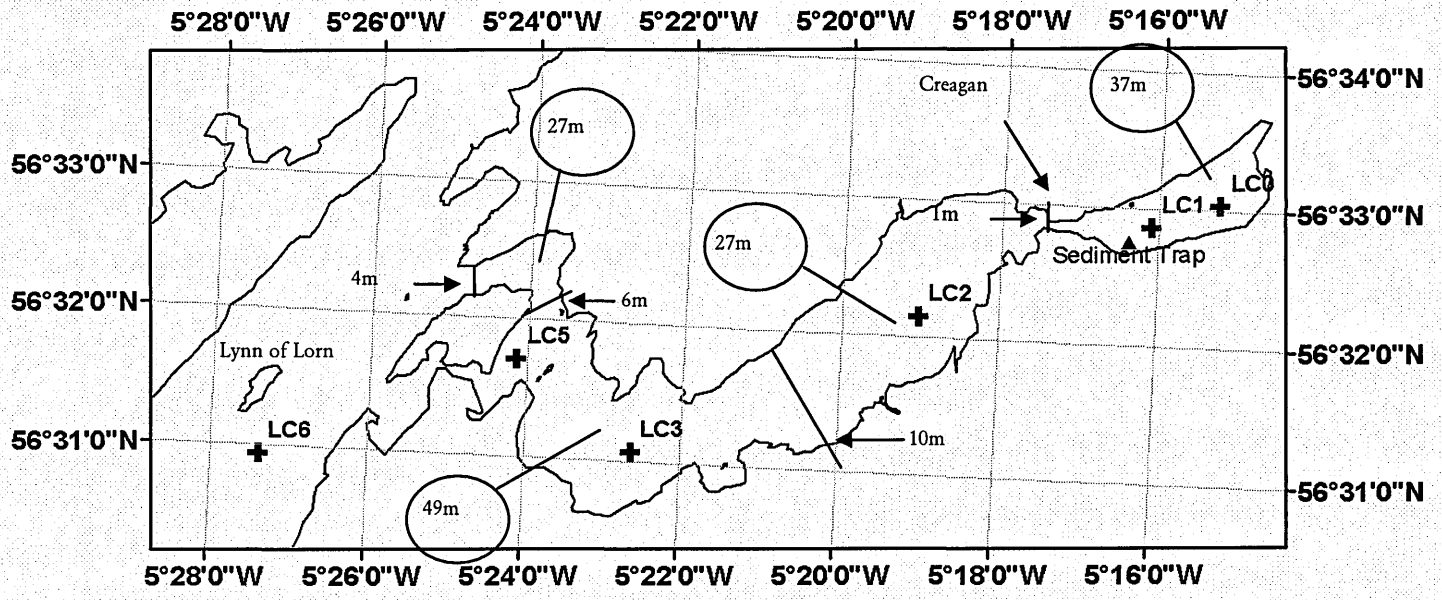

Figure 2.2 Map of Loch Creran. The six sampling locations were, from the head of the loch, LC0, LC1, LC2, LC3 and LC5; LC6 was situated outside Loch Creran. The four sills are shown as lines drawn across the loch, with arrows indicating their depths. Values for the greatest depth for each basin are shown in circles.

2.1.2 Loch Etive

2.1.2.1 Hydrographic conditions

Innermost bottom water stagnates for months or years, with slowly changing temperature and salinity, falling deep oxygen concentration and with a secondary pycnocline* below sill depth. A bottom water renewal is described and shown to be caused by low freshwater runoff. The renewal is a series of overflows of sill water during spring flood tides+ (Edwards and Edelsten, 1977). Replenishment of deep-basin water by ventilation over the sills is spasmodic and controlled by surface stratification; the latter being due primarily to freshwater runoff. In this loch, a limited number of typical brackish-water species occurred uniformly in all the intertidal and shallow subtidal sediments sampled, none of these species occurring in the samples from deeper water. The fauna in Loch Etive is less diverse compared to Loch Creran (Gage, 1972). The upper basin bottom water is renewed aperiodically depending on freshwater input with a mean flushing time of 16 months. Relatively dense overlying water replaces that occupying the deep parts of the basin (Edwards and Edelsten, 1977).

Water above sill level in the deepest basin usually has low salinity because of locally high freshwater runoff. When runoff lessens, salinity and density rise and the loch bottom water is renewed by density current inflow. When runoff rises again, the new water is isolated by its high density and it stagnates for many months (Edwards and Grantham, 1986). The residence time of the isolated waters may extend to 30 months, with a mean of 16 months (Edwards and Trusdale, 1997).

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* **pycnocline** layer in which the density of the water rapidly increases with depth, because of the presence of a halocline or a thermocline or both.

+ **Flood tide** the period in a tidal cycle between low water and the next high water.

Spring tide tidal period with a large tidal range, with the high water and low water both attaining relatively extreme values. Spring tide occurs about once every fortnight, alternates with neap tide after about one week (Baretta-Bekker *et al.*, 1998).

The tidal cycle in Loch Etive shows an attenuation in amplitude and a lag in phase when compared to that just outside the loch in the Firth of Lorne; the mean spring-tide range of 3.2m being reduced by one third and the times of high and low water delayed by about 1.5h (Gage, 1972).

The salinity structure of the main part of the water mass in both basins remains fairly constant, usually varying between 27 and 28 in the deep upper basin with similar or slightly higher values in the lower basin. The surface layers were, however, strongly affected by freshwater runoff. The surface salinity varied from less than 1 after periods of heavy rainfall to more than 26 during the occasional dry spells. The salinity within the layer decreases up the length of the loch, from its entrance to the head (Gage, 1972).

The temperature regime deep in the upper basin of this loch follows that of the lower basin with a lag of approximately one month due to the restricting effect in Bonawe Narrows. Near the surface there is an increase as surface water warms in spring; deeper, a thermocline at 50m separates the sill water from stagnant bottom water at 11.2°C (Edwards and Edelsten, 1977).

2.1.2.2 Geography

Loch Etive opens into Loch Linnhe, an extension of the Firth of Lorne. This is a glacially overdeepened valley, with two main sills, one at the entrance (Connel narrows) and the other at Bonawe. The latter divides the loch into two roughly equal parts. Loch Etive is 29.5km long and consists of six basins separated by sills: the number of sills, and sills and basins' depths are given in Table 2.1 (Edwards and Sharples, 1986). This is a relatively deep loch with the deepest part approximately 145m (Steven J. Gontarek, *pers. comm.*). The rainwater catchment area is 1400km^2 (Wood *et al.*, 1973; Edwards and Edelsten, 1977). In Loch Etive, the Falls of Lora mark the sill at the seaward entrance to the lower basin, which occupies 11.35km^2. Another shallow narrows occurs at about 11km further inland at Bonawe opening into an upper basin with a surface area of 16.94km^2 (Gage, 1972).

There are altogether six sills in Loch Etive. The sills, their depths and the basins' depths are also illustrated in Figure 2.3.

Table 2.1 Sills and basins depth in Loch Etive. The information of the number of sills, the mean depth of each sill and the mean basin depth (Edwards and Sharples, 1986).

Sill no.	1	2	3	4	5	6
Mean depth of sill (m)	7	4	5	8	12	8
Basin depth (m)	42	22	49	38	68	145

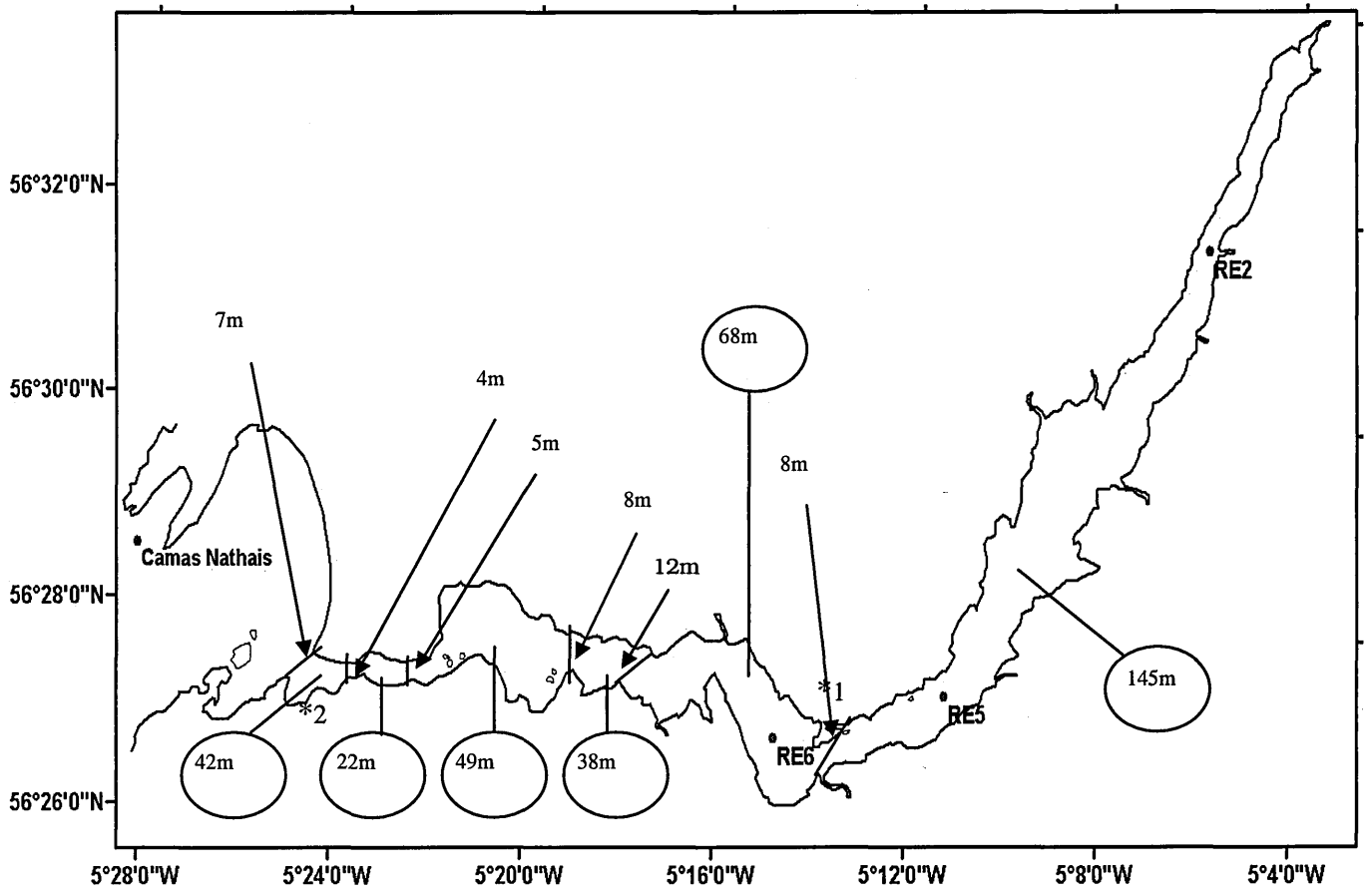


Figure 2.3 Map of Loch Etive. The sills are shown as lines drawn across the loch, with arrows indicating their depths. Values for the greatest depth for each basin are shown in circles. *1=Bonawe, *2=Connel.

2.1.3 Study areas

Loch Creran

River Creran at the head of the loch is the main source of freshwater input into the loch. There were five sampling locations along the loch, and one location situated outside the loch. The coordinates of these locations are given in Table 2.2. There were two sampling stations in the uppermost basin at the head of the loch: LC0 and LC1. This uppermost basin is separated from the next basin by a narrows at the Creagan bridge. In the middle basin were LC2 and LC3. Further to the mouth of the loch was LC5. LC6 was situated outside the loch. The sediment trap was located near LC1 (Figure 2.4).

Table 2.2 Sampling locations in Loch Creran.

Sampling locations	Depth (m)	Latitude (N)	Longitude (W)
LC 0	15.42	56°33.04	05°15.25
LC 1	37	56°32.85	05°16.12
LC 2	27	56°32.14	05°19.04
LC 3	49	56°31.07	05°22.63
LC 5	27	56°31.71	05°24.14
LC 6	48.94	56°30.94	05°27.37
Sediment trap	10	56°32.75	05°16.39

Loch Etive

There were three sampling stations within the loch: RE2, RE5 and RE6. Camas Nathais was situated outside the loch in the Firth of Lorn. The coordinates of these locations are given in Table 2.3. These locations are also illustrated in Figure 2.4. River Etive at the head of the loch and River Awe at Bonawe (before RE6) are the two main freshwater inputs into Loch Etive.

Table 2.3 Sampling locations in Loch Etive.

Sampling locations	Depth (m)	Latitude (N)	Longitude (W)
RE 2	37	56°31.73	05°05.96
RE 5	123	56°27.33	05°11.25
RE 6	57	56°26.87	05°14.77
Camas Nathais		56°28.54	05°28.11

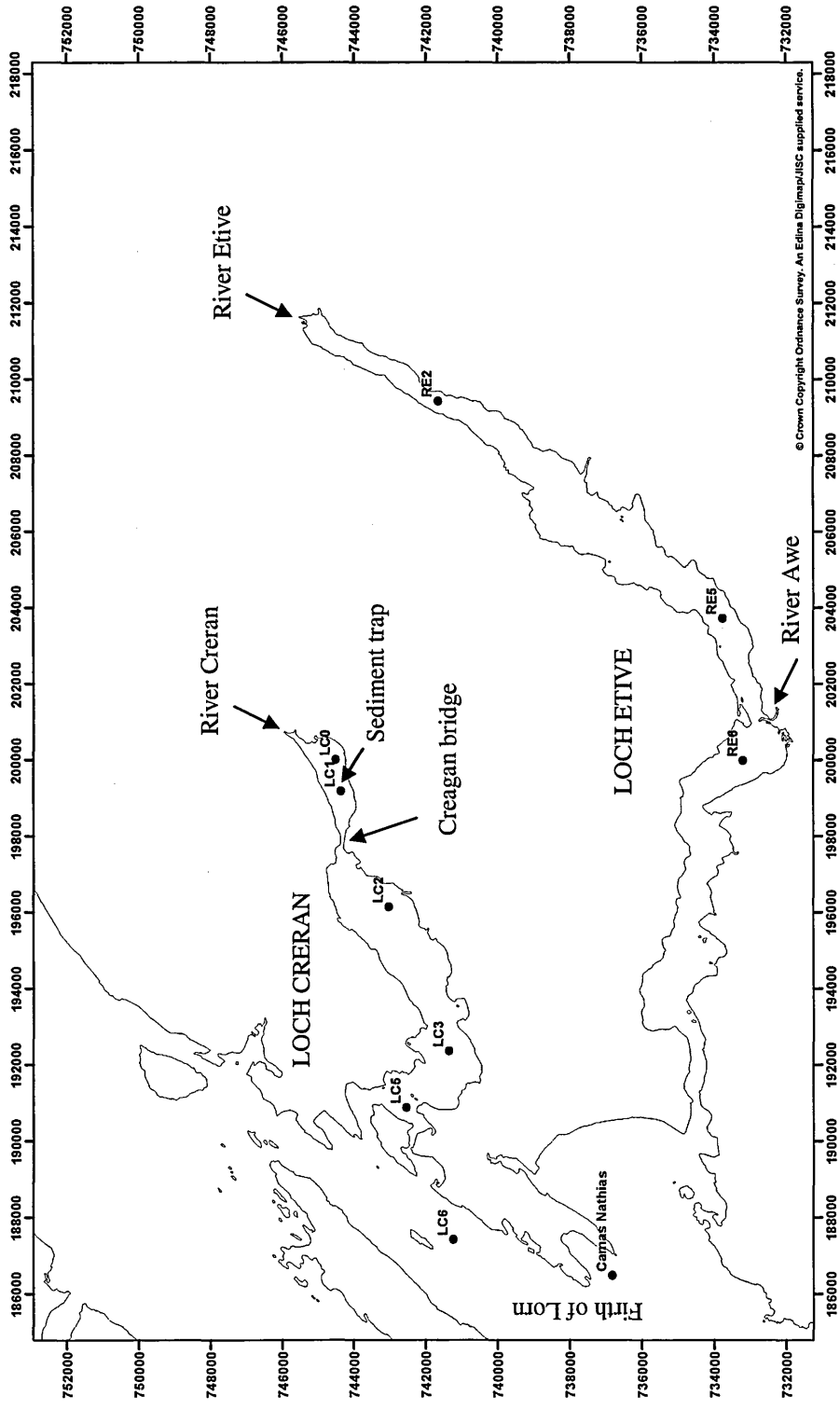


Figure 2.4 Map of study area showing the sampling locations.

2.2 ANALYTICAL METHODS

2.2.1 Sampling and sample pre-treatment

The procedures for the sediment, sediment trap and water sampling are given in this section. Sediment samples were collected using a Craib corer (Craib, 1965) lined with acrylic core tube (24cm long x 5.9cm i.d.) (Figure 2.5). The corer was lowered to the sediment surface in order to obtain sediment cores with undisturbed water-sediment interface layer. In each location, triplicate of sediment cores were obtained; the corer was lowered three times in order to obtain three separate sediment cores. This procedure would be repeated if the sediment cores obtained were found to be disturbed.

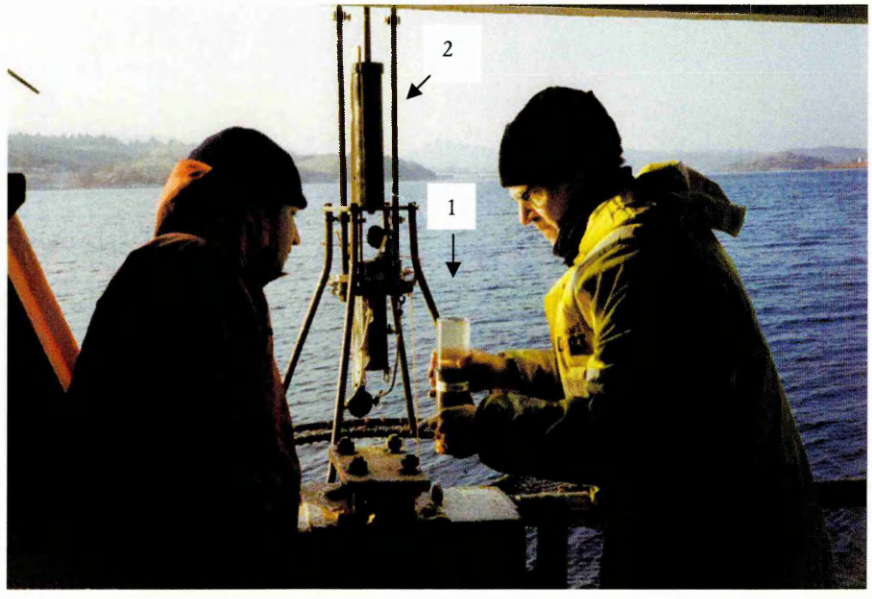


Figure 2.5 Photograph showing sediment sampling. An acrylic core tube (1) with undisturbed water-sediment interface layer being taken out from the Craib corer (2). [Martyn Harvey was holding the core tube. With him was Eric Fouilland]

Sampling times were planned to coincide with neap tides* so that the research vessel "Seol Mara" could cross the shallow water at the Creagan Bridge in Loch Creran. Sampling was carried out initially in Loch Etive for three months and followed by sampling in Loch Creran for 20 months. The sampling dates and locations are given in Table 2.4. In both lochs, the stations were chosen to provide a range of sediment samples to reflect transition from samples dominated by riverine input to stations dominated by marine input. RE6 in Loch Etive and LC1 in Loch Creran were visited on a monthly basis, enabling the effect of seasonal variations to be monitored. The other locations were visited on a rotational basis with one another throughout the sampling time.

Table 2.4 Timetable for monthly sampling. * = sampling months where the 0-1cm and 9-10cm sediment layers were studied. In a normal month, only the surface 0-1cm sediment layer was studied (see Chapter 3). X = sample was taken from the sediment trap that month.

Date	Sampling sites		
	Monthly basis	Other stations sampled	Sediment trap
17.1.2001.	RE6 *	RE5	
14.2.2001.	RE6	RE2	
20.3.2001.	RE6	Camas Nathais	
29.5.2001.	LC1 *	LC2	
2.7.2001.	LC1	LC3	
16.7.2001.	LC1	LC0	
29.8.2001.	LC1 *	LC5	
28.9.2001.	LC1	LC6	
24.10.2001.	LC1	LC2	X
12.12.2001.	LC1*	LC3	X
8.1.2002.	LC1	LC0	X
6.2.2002.	LC1	LC5	X
7.3.2002.	LC1*	LC2 & LC0	X
21.3.2002.		LC3, LC5 & LC6	X
4.4.2002.	LC1	LC2 & LC0	X
7.5.2002.	LC1	LC3	X
4.6.2002.	LC1*	LC0	X
2.7.2002.	LC1	LC5	X
1.8.2002.	LC1	LC6	X
2.9.2002.	LC1*	LC2*	X
1.10.2002.	LC1	LC3*	X
14.11.2002.	LC1	LC0	X
12.12.2002.	LC1*	LC5*	X

* **neap tide** tidal period during which the tidal range is small, the high water being lower and the low water being higher than normal (Baretta-Bekker *et al.*, 1998).

In the laboratory, firstly the oxygen uptake measurements from intact sediment cores were carried out (section 2.2.4). These procedures of samples pre-treatment were then carried out. The sediment was sliced to 1cm thickness down the whole core and the sediment slices kept in containers (Figure 2.6). The containers, with sediments, were put into freezer (-20°C) overnight. The next day, the sediments were subjected to freeze-drying. The dried sediments were then ground to fineness with a mortar and pestle and ready for the subsequent experiments: lignin analysis, loss on ignition, carbon and nitrogen (CN), stable carbon isotopic and phosphate analyses.

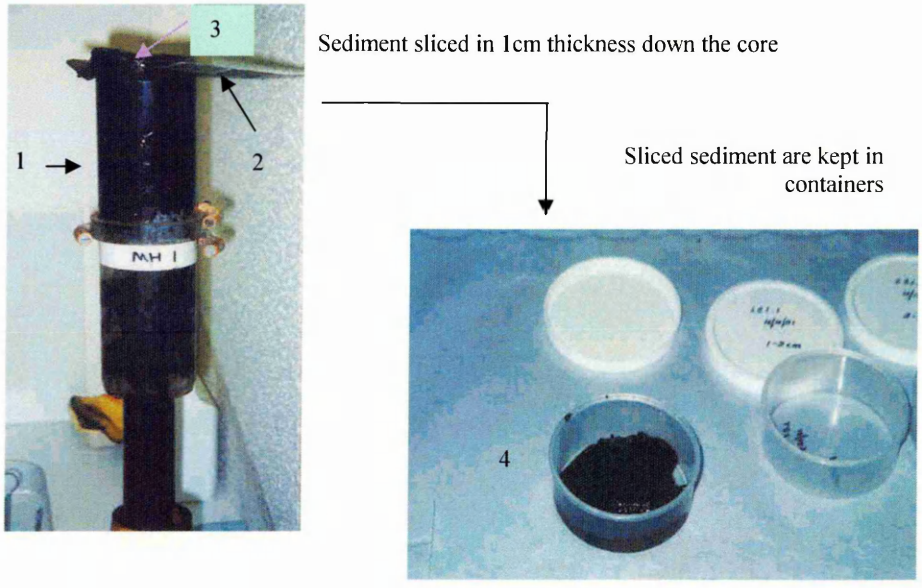


Figure 2.6 Sediment sample preparation. Sediment was sliced from a core tube (1) using a thin aluminium plate (2) and a '1cm' thick ring (3) for measurements purpose. Sliced sediment was kept in plastic container (4) in the freezer overnight and then subjected to freeze-drying.

2.2.1.1 Sediment sampling at Creran Head

Sediment samples were also collected from Creran Head (56°33.52N, 5°14.50W), a location where the River Creran ends and Loch Creran begins. This location was accessed by foot. Sediments were collected by pressing the core tubes into the sediment, and these core tubes were

Sediments were collected by pressing the core tubes into the sediment, and these core tubes were taken out carefully with minimal disturbance to the sediments. In the laboratory the sediments were treated as stated previously, and then subjected to various experimental analyses.

2.2.1.2 Sediment traps

Sediment traps were deployed near LC1 at 10m below the surface. Overnell and Young (1995) found that of the total depth of 110m, the 20m below surface water provides a good estimate for the net sedimentation rate. As the depth of the water column where the sediment traps were deployed in this study was approximately 35m, the depth below 10m of the surface water would provide a good representative.

A picture of the traps is shown in Figure 2.7. Each of the tubes of the traps is of 11cm i.d. and 11m long and has a removable clear plastic collecting tubes. In the laboratory, the collecting tubes were allowed to stand until all sediment had settled down. Water was then siphoned out, the sediment slurry decanted into centrifuge tubes and centrifuged. The remaining water decanted from the centrifuge tubes and the sediments remaining in the tubes were put into freezer overnight and subjected to freeze-drying the next day. The dried sediments were then ready for subsequent analyses.

The sediment trap materials were also used to calculate the monthly sedimentation rate. Sedimentation rate was calculated as follows:

Surface area of tube $= \pi (0.11m/2)^2 = 0.0095m^2$

Surface area of 4 tubes $= 0.0095m^2 \times 4 = 0.038m^2$

Sedimentation rate = dry weight of sediment collected in the four tubes/$0.038m^2$/total day of trap deployment

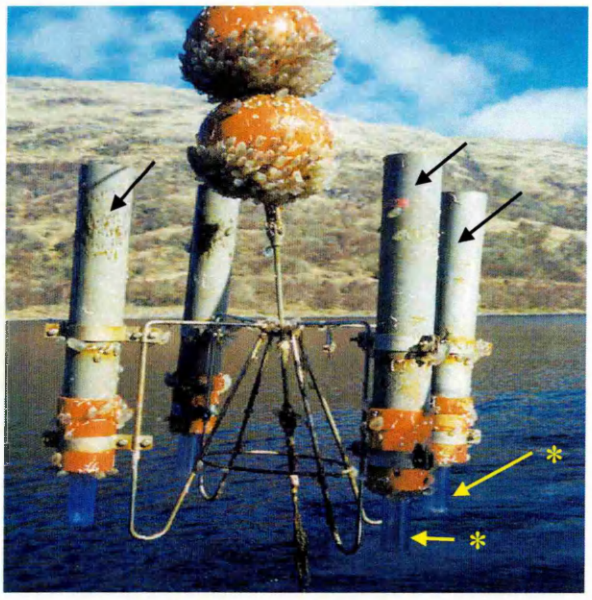


Figure 2.7 Sediment traps. The arrows show the tubes of the traps. The collecting tubes (*) were collected and changed on a monthly basis.

2.2.1.3 Water sampling from River Creran

Water sampling was carried out from a bridge situated further away from the mouth of River Creran. The location of this bridge is 56°34.27N, 5° 13.52W.

Water sample was collected by lowering a bucket over a bridge (Figure 2.8). In the laboratory water samples were treated as stated in section 2.2.3.8. During every sampling trip, the river flow rate was roughly estimated using "pooh sticks" (Milne, 1966). A stick was thrown onto the water from above the bridge at 1m intervals across the bridge. The time for the stick to travel the width of the bridge was also recorded. Flow rate was calculated by dividing the width of the bridge with the time travelled by the stick.

Figure 2.8 Water sampling from River Creran. Water was collected by lowering a bucket over a bridge onto the water (as shown by the arrow). Water collected from the bucket was then poured into plastic containers, and transported back to the laboratory.

2.2.2 Lignin analysis

Lignin is a high molecular weight phenolic polymers occurring in the woody tissues of vascular plants and in soil and sediment organic matter. Sample is treated with the alkaline CuO oxidation process to produce simple lignin-derived phenols to be analysed by gas chromatography (Hedges and Ertel, 1982). Methods followed for the CuO oxidation process were well established by Hedges and Ertel (1982), Readman *et al.*, (1986) and Goni and Hedges (1992). A summary of the CuO oxidation method used in this research is also given by Loh *et al.* (2002).

Approximately 0.5g of dried sediment sample (Readman *et al.*, 1986) was precisely weighed into a PTFE-lined bomb and 1.0g CuO powder added into the bomb. Seven ml of 8% w/v aqueous NaOH in test tube was bubbled with nitrogen gas for about 5 minutes (Hedges and Ertel, 1982) in order to remove oxygen. The bomb and test tubes were loaded into a glove bag 'Cheltenham PA

X-27-27H' (Figure 2.9) which was then purged with nitrogen for about 15 minutes, during which the volume was flushed with nitrogen several times to remove air, enabling the CuO oxidation to be conducted under oxygen free conditions. This is to prevent superoxidation from occurring (Hedges and Ertel, 1982). The NaOH solution was then added into the bomb and the bomb sealed.

The bomb was then placed in a 'series 104 chromatograph' oven and heated from room temperature to 155°C for three hours (Goni and Hedges, 1992). The bomb was manually shaken every hour for 30 seconds. After 3 hours, the bomb was cooled rapidly under running water. Contents of the bomb were then transferred with 8% w/v $NaOH/H_2O$ washings to a centrifuge tube. Residual sediments were centrifuged at 600g x 10min. Residue was rinsed twice with 20ml NaOH solution and centrifuged as before. Supernatants were combined and acidified to pH1 with approximately $30cm^3$ of 6M HCl, and 100 μg ethyl vanillin added into the supernatant as the internal standard.

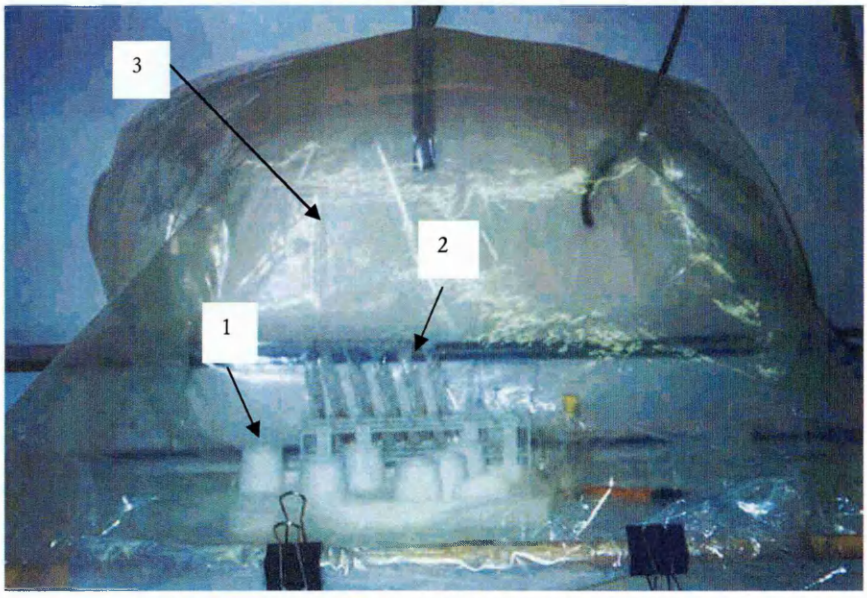
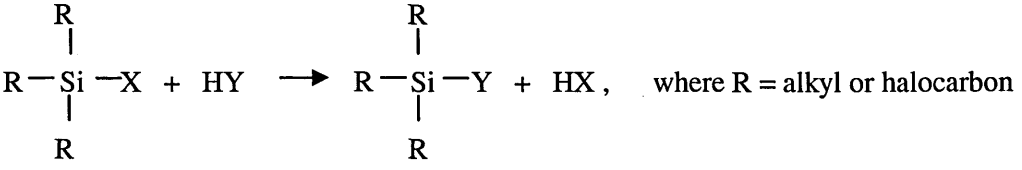


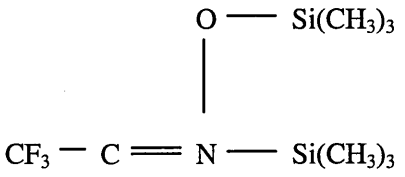
Figure 2.9 Glove bag with its contents. The bombs (1) with their caps, along with NaOH solution in test tubes (2) were all kept in the glove bag (3) for nitrogen purging. Sediment samples and the cupric oxide powder were inside the bombs.

The product was extracted three times with diethyl ether (3 x $10cm^3$), the extracts combined, dried with anhydrous Na_2SO_4, filtered through 'Whatman GF/F', and then rotary evaporated to near dryness and the oxidation product transferred into a vial with the diethyl ether using a pipette. A 'Pierce model 18780 Reacti-Vap TM' evaporating unit was then used to remove the ether by nitrogen blowing down. The diethyl ether used in the extraction process was previously treated with $Fe(NH_4)_2(SO_4)_2.6H_2O$ to remove peroxides.

A derivatisation step is required for the CuO oxidation products prior to analysis. Silylation is the most widely used derivatization procedure for sample analysis by gas chromatography. The purpose of silylation is to change the properties of a molecule from polar and active to non-polar and inert, which is stable for GC analysis. The term silylation includes all reactions of this type:



One example of silylation or derivatisation is the formation of trimethylsilyl (TMS) ethers where R = methyl. The silylation reagent used here is the bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 10% trimethylchlorosilane (TMCS) (Sigma Aldrich) as catalyst. Their structures are shown here (Poole, 1979):



bis(trimethylsilyl)trifluoroacetamide (BSTFA)

and,

$(CH_3)_3SiO$: Trimethylchlorosilane (Abbreviation: TMCS)

For the silylation purpose, the oxidation product was dissolved in 100µl dried toluene. An equal volume of 100µl of the BSTFA/TMCS was added to each sample in a nitrogen-purged glove bag. Samples were then heated at 90°C (Wilson *et al.*, 1985) for 10 minutes using a 'Techne Dri-Block DB.3D' heater. The silylated samples were then allowed to cool down and analysed immediately by gas chromatography (GC). Exactly 2.0µL of sample was drawn into the syringe, followed by 3.0µL of air and injected into the GC. The action of sample injection into the GC was always the same; for example, the action and timing where the syringe was pulled and injected into the GC were the same. Also, no air bubble was trapped in the syringe barrel between the needle and the sample plug.

Gas chromatography (GC) is a method for separating components of mixtures of volatile compounds in order to identify and determine the quantity of each component. The sample is introduced into the GC through a sample injector. Carrier gas elutes the components of the mixture through the column, and the separated components are detected at the far end by a detector (Littlewood, 1970) after different retention times. Chromatogram is produced by continuous registration of electrical signals generated by the detector. The peak area and peak height give information on the amount of the corresponding eluted component (Harvey, 2000).

In the GC application in this study, the injected sample was split with a ratio 100:1, indicating that only one hundredth of the injected sample will pass through the column and be detected. The sample was introduced through a septum into the carrier gas flow, vaporized and mixed with the carrier gas. The carrier gas flow was then divided into two parts, with a variable flow ratio, the smaller part of the carrier gas-sample mixture enters the column, whereas the larger flow by-passes the column inlet and leaves the system (Schomburg, 1990).

In GC with flame ionization detector (GC-FID), the signal is considered to be proportional to the concentration of the compound being burnt in the flame so that the area under the peak reflects the amount of the compound (Rouessac and Rouessac, 1998). In GC with mass spectrometer (GC-MS), different ion masses reach and are detected by the detector, and after calibration this

provides molecular mass and amounts of each ion formed. The mass-spectra are used to identify the chemical nature of the compound (McMaster and McMaster, 1998).

Lignin phenols identification was carried out using a gas chromatograph-mass spectrometer (GC-MS TRACE MS Thermo Quest, Finnigan) fitted with a 0.25mm i.d. x 30m of 5% phenyl-methylpolysiloxane capillary column (RTX-5MS, RESTEK CORP.) with a split ratio 100:1. Results were integrated and the trimethylsilated CuO oxidation products of the lignin-derived phenols were identified using 'Xcalibur' software. The initial temperature was 100°C, increasing at 5°C per minute to 200°C. This was held for 10 minutes. For the second ramp, this temperature increased at 20°C per minute to 300°C. The inlet temperature was 300°C, the oven maximum temperature was 350°C and the equilibrium time was 0.5 minutes.

Quantification of lignin phenols individual monomers was accomplished by a PERKIN-ELMER 8410 GC-FID fitted with a 0.25mm i.d. x 30m of 100% dimethylpolysiloxane (ZB-1, Phonomenex, Zebron) column and a split ratio 100:1. Flame ionization detector output was collected and integrated using the 'Star Chromatograph Workstation' software. The initial temperature for the first ramp was 100°C and increased at a rate of 5°C per minute. For the second ramp, the temperature was 200°C and increased at 20°C per minute to 300°C, and this was held for 5 minutes. Both injector and detector temperatures were 300°C. The equilibration time was 2 minutes. The injected sample was split with a ratio 100:1.

Figure 2.10 shows a simplified diagram of the procedures involved in the elucidation and determination of lignin components from sediment samples, and also from the dissolved and particulate matter from water samples (Loh *et al.*, 2002).

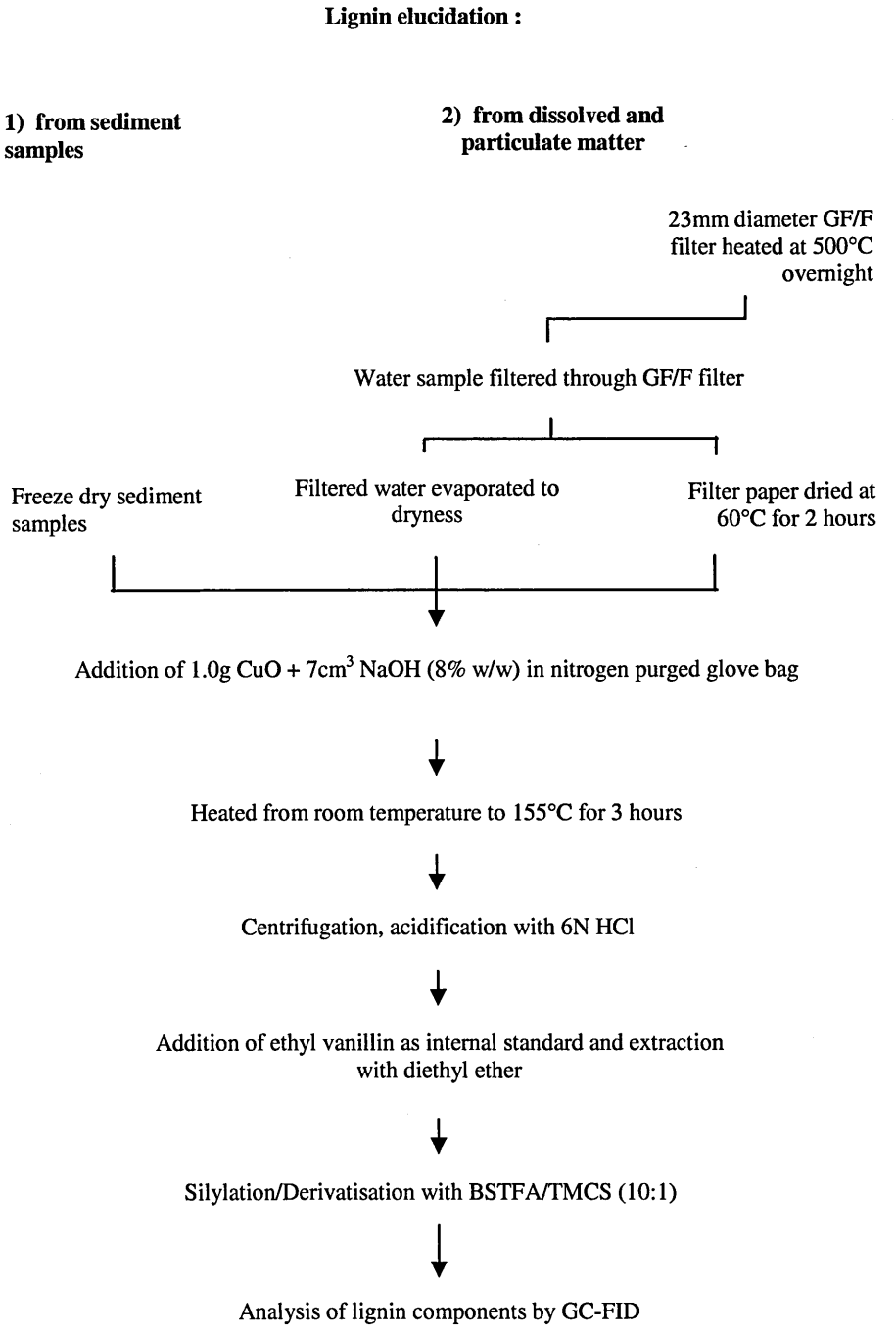


Figure 2.10 Diagram showing summary of the CuO oxidation process.

2.2.3 Lignin analysis validation experiments

Method validation for the lignin analysis consisted of several steps: identification and confirmation of the lignin-derived phenols, followed by determination of the precision and reproducibility of the GC and finally method validation for each procedure of the cupric oxide oxidation method.

2.2.3.1 Identification of lignin-derived phenols

There are altogether 12 lignin phenol standards: p-hydroxybenzaldehyde, p-hydroxyacetophenone, vanillin, ethyl vanillin, acetovanillone, p-hydroxybenzoic acid, syringaldehyde, acetosyringone, vanillic acid, syringic acid, p-coumaric acid and ferulic acid. Ethyl vanillin is used as the internal standard, as it is not found in plants, hence is not one of the lignin oxidation products of the sediment samples. Also it is elucidated within the range of the other 11 lignin phenols as the fourth component (Figure 2.9).

A lignin-standard mixture was made up. Approximately 5mg of each lignin phenol standard (Sigma Aldrich) in powder form was added with diethyl ether to 10ml. 100µl of each standard was mixed with an equal volume of the silylating agent (BSTFA:TMCS), silylated and analysed by GC. The GC conditions were adjusted to give optimum separation of each lignin phenol peak. Retention times of the trimethylsilylated (Me_3Si) lignin-derived phenols were determined by injecting a single Me_3Si-derivatised lignin phenol standard to the GC-FID, and identified by injecting the same sample to a GC-MS. For example, a vanillin standard was injected into the GC-FID, and its retention time determined. The same standard was then introduced into the GC-MS to allow confirmation of its identity. Each lignin-derived phenol standard had its retention time confirmed in this way. After that, a standard solution consisting of a mixture of all 12 lignin phenol standards was made up, silylated and analysed using the GC-FID. The chromatogram obtained is shown in Figure 2.11.

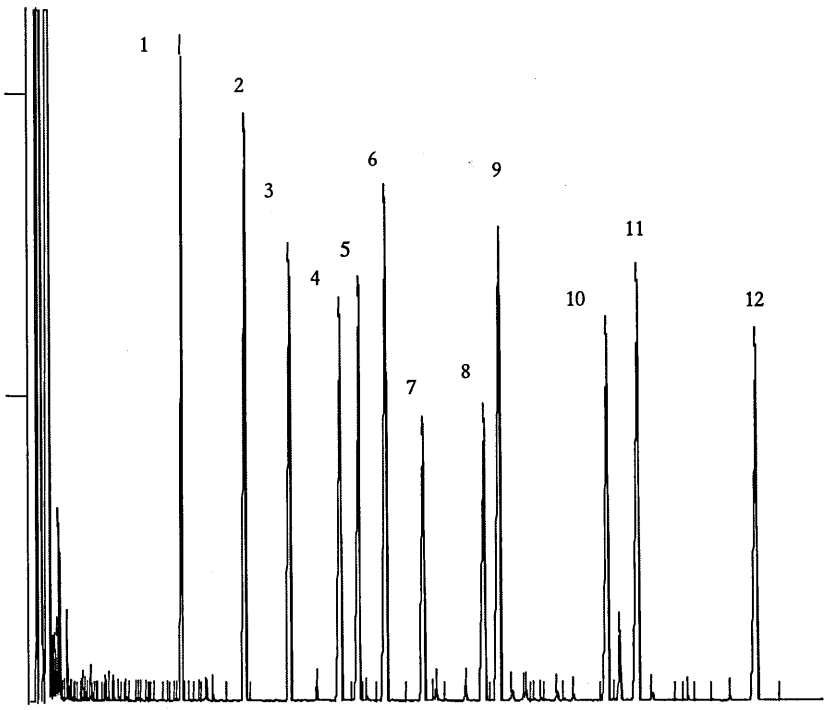


Figure 2.11 Chromatogram of the lignin phenol standards. The 12 Me_3Si-silylated phenols of the standard mixtures was analysed using the gas chromatograph with flame ionization detector (GC-FID). Peaks of each lignin phenol are numbered according to their elution times: 1)p-hydroxybenzaldehyde; 2)p-hydroxyacetophenone; 3)vanillin; 4)ethyl vanillin; 5)acetovanillone; 6)p-hydroxybenzoic acid; 7)syringaldehyde; 8)acetosyringone; 9)vanillic acid; 10)syringic acid; 11)p-coumaric acid; 12)ferulic acid. Quantification of these phenols was carried out with a PERKIN-ELMER 8410 gas chromatograph with flame ionization detector fitted with a 0.25mm i.d. x 30m of 100% dimethylpolysiloxane and split ratio 100:1. Initial temperature was 100°C and increased at 5°C/minute to 200°C; then increased at 20°C/minute to 300°C and held for 5 minutes. Both injector and detector temperatures were 300°C. Equilibration time was 2 minutes.

2.2.3.2 Gas chromatography precision/reproducibility

Reproducibility of the GC-FID lignin phenols retention times

Reproducibility of the GC-FID lignin phenols retention time was determined by injecting the same amount (i.e. same volume of the same concentration) of the same sample of lignin standards mixture into the GC several times and the results are given in Table 2.5. Retention times for each lignin did not deviate significantly and were reproduced to a high precision. There was no overlap between the ranges for different peaks. Also the percentages reproducibility was good. The % reproducibility is calculated as:

Reproducibility = (б/n) x 100% (and this is also known as the coefficient of variation); where б is standard deviation and n is mean.

Table 2.5 Lignin phenols and their retention times.

(a) Trial 1: Retention times (mean ± standard deviation) and reproducibility for all 12 lignin-derived phenols in a standard mixture, as detected by the GC-FID, n=8

No	Lignin	Retention times									
		1	2	3	4	5	6	7	8	Mean±SD	CV (%)
1	p-Hb	4.95	5.015	4.938	4.821	5.009	5.072	4.995	4.966	4.971 ±0.074	1.49
2	p-Ha	6.513	6.599	6.499	6.389	6.602	6.668	6.586	6.536	6.549 ±0.085	1.30
3	Vanillin	7.617	7.674	7.59	7.465	7.677	7.752	7.662	7.624	7.633 ±0.084	1.10
4	EV	8.811	8.868	8.813	8.689	8.879	8.944	8.879	8.808	8.836 ±0.076	0.86
5	Acetovan	9.321	9.339	9.296	9.156	9.321	9.514	9.31	9.297	9.319 ±0.097	1.04
6	p-Hba	9.94	9.995	9.905	9.814	10.029	10.115	10.023	9.957	9.972 ±0.091	0.91
7	Syringal	10.901	10.969	10.872	10.73	10.965	11.061	10.95	10.922	10.921 ±0.096	0.88
8	Acetosyr	12.457	12.579	12.373	12.203	12.581	12.699	12.564	12.529	12.498 ±0.153	1.22
9	Va	12.7	12.769	12.676	12.535	12.763	12.856	12.754	12.725	12.722 ±0.093	0.73
10	Sa	15.288	15.312	15.259	15.153	15.321	15.385	15.302	15.284	15.288 ±0.066	0.43
11	p-Cou	16.063	16.089	16.029	15.913	16.102	16.165	16.077	16.056	16.062 ±0.072	0.45
12	Fe	18.741	18.735	18.726	18.649	18.753	18.785	18.732	18.726	18.731 ±0.038	0.20

Abbreviations: p-Hb, p-hydroxybenzaldehyde; p-Ha, p-hydroxyacetophenone, EV, ethyl vanillin; Acetovan, acetovanillone; p-Hba, p-hydroxybenzoic acid; Syringal, syringaldehyde; Acetosyr, acetosyringone; Va, vanillic acid; Sa, syringic acid; p-Cou, p-coumaric acid; Fe, ferulic acid. 'CV' is the coefficient of variation, calculated as the percentage standard deviation divided by the mean; 'SD' is the standard deviation.

(b) Trial 2: Retention times (mean ± standard deviation) and reproducibility for all 12 lignin-derived phenols in a standard mixture, as detected by the GC-FID, n=3.

No	Lignin	Retention times					
		1	2	3	Mean±SD		CV (%)
1	p-Hb	4.817	4.711	4.866	4.798	±0.079	1.65
2	p-Ha	6.357	6.243	6.416	6.339	±0.088	1.39
3	Vanillin	7.453	7.329	7.503	7.428	±0.090	1.21
4	EV	8.672	8.547	8.725	8.648	±0.091	1.05
5	Acetovan	9.194	9.9	9.206	9.433	±0.404	4.28
6	p-Hba	9.755	9.616	9.816	9.729	±0.103	1.06
7	Syringal	10.714	10.576	10.763	10.684	±0.097	0.91
8	Acetosyr	12.264	12.06	12.315	12.213	±0.135	1.11
9	Va	12.505	12.363	12.573	12.480	±0.107	0.86
10	Sa	15.102	14.993	15.157	15.084	±0.083	0.55
11	p-Cou	15.895	15.752	15.945	15.864	±0.100	0.63
12	Fe	18.735	18.606	18.698	18.680	±0.066	0.35

From Table 2.5a, the CVs range from 0.20 to 1.49%. From Table 2.5b, the CVs range from 0.35 to 4.28%. Hence the CV for the GC-FID retention times ranged from 0.20 to 4.28%.

Reproducibility of lignin phenol concentrations

The concentration of lignin phenols was calculated based on the peak area ratio of the lignin phenol to the peak area of the internal standard ethyl vanillin:

$$\text{Conc y} = \frac{\text{Peak area of lignin phenol y}}{\text{Peak area of ethyl vanillin}} \times \text{Actual concentration of ethyl vanillin}$$

Data from repeated injections of the same lignin phenol standards mixture are presented in Table 2.6. The original total lignin concentration for all of the lignin phenol standard concentrations is labelled as "O" in Tables 2.6 (a and b). It was found that the total lignin for all the replicates of the detected concentrations was almost the same as the original total lignin concentration.

Table 2.6 Lignin phenols and their concentrations.

(a) Trial 1: Concentrations (mean ± standard deviation) for all lignin derived phenols and their reproducibility; n=8.

No	Lignin	Detected concentration (mg/ml diethyl ether)										
		"O"	1	2	3	4	5	6	7	8	Mean±SD	CV (%)
1	p-Hb	0.0531	0.089	0.078	0.067	0.061	0.064	0.095	0.068	0.091	0.077 ±0.013	16.88
2	p-Ha	0.0542	0.081	0.089	0.062	0.073	0.074	0.106	0.073	0.088	0.081 ±0.013	16.05
3	Vanillin	0.0517	0.071	0.064	0.052	0.051	0.052	0.076	0.052	0.073	0.061 ±0.011	18.03
4	EV	0.0509	0.051	0.051	0.051	0.051	0.051	0.051	0.051	0.051	0.051 0	0
5	Acetovan	0.0506	0.079	0.052	0.056	0.052	0.037	0.078	0.038	0.056	0.056 ±0.016	28.57
6	p-Hba	0.0522	0.088	0.078	0.059	0.089	0.083	0.116	0.081	0.095	0.086 ±0.016	18.60
7	Syringal	0.0504	0.061	0.053	0.044	0.044	0.044	0.063	0.043	0.061	0.052 ±0.009	17.31
8	Acetosy	0.0527	0.052	0.056	0.021	0.046	0.051	0.088	0.048	0.063	0.053 ±0.019	35.85
9	Va	0.0505	0.08	0.057	0.058	0.061	0.044	0.054	0.045	0.067	0.058 ±0.012	20.69
10	Sa	0.0539	0.065	0.053	0.047	0.047	0.044	0.061	0.042	0.062	0.053 ±0.009	16.98
11	p-Cou	0.0522	0.081	0.062	0.057	0.064	0.053	0.069	0.049	0.071	0.063 ±0.010	15.87
12	Fer	0.0501	0.017	0.014	0.014	0.014	0.013	0.015	0.011	0.018	0.015 ±0.002	13.33
	Total	0.622	0.815	0.705	0.589	0.652	0.610	0.873	0.601	0.794		18.18 (mean CV)

"O" means the original concentration of the lignin standard prepared for the analysis. Lignin standard was made up by dissolving 0.5mg of each standard with 10ml diethyl ether. There were altogether 12 lignin standards, hence resulting in 6mg/10ml or 0.6mg/ml diethyl ether. The total lignin obtained here were about 20% to the total lignin is 1mg/g, with a mean of about 30% to the original value.

(b) Trial 2: Concentrations for all lignin derived phenols and their reproducibility; n=3.

No.	Lignin	Detected concentration (mg/ml diethyl ether)						
		"O"	1	2	3	mean	±SD	CV (%)
1	p-Hb	0.0503	0.060	0.059	0.061	0.060	±0.001	1.67
2	p-Ha	0.0505	0.061	0.061	0.066	0.062	±0.003	4.84
3	Vanillin	0.0526	0.053	0.053	0.053	0.053	±0	0
4	EV	0.0501	0.050	0.050	0.050	0.050	±0	0
5	Acetovan	0.0519	0.059	0.057	0.053	0.056	±0.003	5.36
6	p-Hba	0.0509	0.070	0.069	0.076	0.072	±0.004	5.56
7	Syringal	0.0514	0.042	0.043	0.042	0.042	±0.001	2.38
8	Acetosyr	0.0549	0.050	0.048	0.042	0.047	±0.004	8.51
9	Va	0.0522	0.062	0.061	0.066	0.063	±0.003	4.76
10	Sa	0.0537	0.052	0.049	0.054	0.052	±0.003	5.77
11	p-Cou	0.0578	0.076	0.074	0.072	0.074	±0.002	2.70
12	Fe	0.0573	0.063	0.056	(0.033)	0.059	±0.005	8.47
	Total	0.6336	0.697	0.680	0.668			4.17 (mean CV)

"O" means the original concentration of the lignin standard prepared for the analysis. The data in the bracket is not used in the calculation of mean, standard deviation (SD) and coefficient of variation (CV), as this results in a huge CV.

From Table 2.6a, the CVs ranged from 13.33 to 35.85%. From Table 2.6b, the CVs range from 0 to 8.51%. Hence the reproducibility (CV) of the GC-FID lignin phenols concentrations ranges from 0 to 35.85%. These values are within the ranges of reproducibility values reported from past studies: Hedges and Parker (1976), Hedges *et al.* (1982, 1985), and Wilson *et al.* (1985). Trial 2 has better reproducibilities than Trial 1, most probably because samples in Trial 2 were analysed immediately. This prevents loss of samples due to evaporation.

2.2.3.3 Detection limit

The detection limit of the GC-FID was determined using different concentrations of lignin phenol standards. Detection limit is the minimum detectable quantity (Skoog *et al.*, 1996). Concentration range for the lignin phenol standards mixture injected into the GC were: 5mg, 1mg, 0.1mg, 0.05mg and 0.01 in 10mls diethyl ether; each standard was diluted from the previous highest concentration as follows. The stock solution was 5mg/10ml of lignins in diethyl ether. The 1mg/10ml standard concentration was diluted from the 5mg/10ml stock solution; the 0.1mg/10ml solution was diluted from the 1mg/10ml solution, and so on. The standard solution used here was a mixture of all 12 lignin phenol standards: each lignin phenol was precisely weighed to approximately 5.0mg and all of them mixed and dissolved in 10ml of diethyl ether. 100µl of the 5mg/10 ml standard solution was mixed with 100µl of BSTFA:TMCS (10:1) and silylated at 90°C for 10 minutes, and ready for GC analysis.

Detection limit was 0.01mg/10ml diethyl ether (corresponding to 0.001mg) for vanillin, ethyl vanillin, acetovanillone, syringaldehyde and acetosyringone. The detection limit was 0.05mg/10ml diethyl ether (or 0.005mg) for p-hydroxybenzaldehyde, p-hydroxyacetophenone, p-hydroxybenzoic acid, p-coumaric acid and ferulic acid; whilst for vanillic acid the detection limit was 1mg/10ml diethyl ether or 0.1mg (Table 2.7a). Detection limits were then calculated in µg and µM (Table 2.7b).

Hedges and Ertel (1982) reported a minimum detectability of about 0.1μg of an individual phenol in an oxidation product mixture. Wilson *et al.* (1985) found the detectability unit of their analytical procedure as approximately 10μg of lignin in 1g dry weight of sediment. The detectability unit found here was 0.002 to 0.01μg/g. Dittmar and Lara (2001) found the detection limit for the individual phenols ranged between 70 and 200nM. This means that the lignin phenols are detected to a lower minimum values compared to Hedges and Ertel (1982) and higher than Wilson *et al.* (1985); within the range from past studies.

The detection limits are also calculated in μM (Table 2.7b) in order to compare with results of Dittmar and Lara (2001). Here is an example of a calculation of the detection limit in molarity (M) for 4-hydroxybenzaldehyde (MW 194.21):

Detection limit for p-hydroxybenzaldehyde (p-hb) was 0.05mg/10ml

0.05 mg of p-hb $\longrightarrow$ 10ml

0.05 μg of p-hb $\longrightarrow$ 10μl

2 μl of the standard was injected into the GC, this is equivalent to 0.01μg p-hb.

0.01 μg $\longrightarrow$ 2 μl

$$\text{Moles of p-hydroxybenzaldehyde} = \frac{0.01\mu g}{194.21}$$

$$\text{Molarity of p-hydroxybenzaldehyde} = \frac{\frac{0.01\ \mu g}{194.21}}{2 \times 10^{-6}\ L}$$

$$= 25.75\ \mu M\ (\mu mol/L)$$

$$= 25750\ nM$$

Table 2.7 Detection limits.

(a) Detection limits for lignin-derived phenols.

No	Lignins	Actual conc.	Concentrations as calculated based on the internal standards and actual concentrations			
		5 mg/10ml	5mg/10ml	1mg/10ml	0.05mg/10ml	0.01mg/10ml
1	p-Hydroxybenzaldehyde	5.66	6.14	1.0290	0.0566	0.0029
2	p-Hydroxyacetophenone	5.81	6.70	1.3133	0.0581	0.0020
3	Vanillin	5.49	5.43	1.0642	0.0549	0.0075
4	Ethyl vanillin	5.09	5.09	1.0180	0.0509	0.0102
5	Acetovanillone	5.13	5.50	1.1053	0.0513	0.0172
6	p-Hydroxybenzoic acid	5.48	5.59	1.5886	0.0548	
7	Syringaldehyde	5.77	4.91	1.0052	0.0577	0.0183
8	Acetosyringone	5.63	4.58	1.0465	0.0563	0.0231
9	Vanillic acid	4.45	4.23	1.0740	0.0445	
10	Syringic acid	4.60	4.97	0.9392	0.0460	
11	p-Coumaric acid	6.24	8.51	1.5870	0.0624	
12	Ferulic acid	4.82	5.42	0.8422	0.0482	

The concentrations of the other 11 lignin-derived phenols were calculated as a ratio of the concentration and peak area of the internal standard ethyl vanillin.

(b) Detection limit for lignin phenols in μg and μM.

Lignin phenols, with molecular weight in bracket		Detection limit	
		μg	Molarity (μM)
1	4-Hydroxybenzaldehyde (194.21)	0.01	25.75
2	4-Hydroxyacetophenone (208.24)	0.01	24.01
3	Vanillin (224.24)	0.002	4.46
4	Ethyl vanillin (238.27)	0.002	4.20
5	Acetovanillone (238.27)	0.002	4.20
6	4-Hydroxybenzoic acid (282.3)	0.01	17.71
7	Syringaldehyde (254.27)	0.002	3.93
8	Acetosyringone (268.29)	0.002	3.73
9	Vanillic acid (312.33)	0.01	16.01
10	Syringic acid (342.35)	0.01	14.60
11	p-Coumaric acid (308.34)	0.01	16.22
12	Ferulic acid (338.37)	0.01	14.78

Detection limit in μg is obtained from Table 2.6a. Detection limit in μM is calculated from the former; see text for calculation. The formula weight (in bracket) is the formula weight of the trimethylsilated structure of the phenol.

The minimum concentrations used in the calculation of the detection limits in μg and μM, are the actual concentrations diluted from the stock solution, not the detected concentrations from the GC. This is in order to obtain standardization for individual lignin phenols, and so that it is easier to read and calculate. Also the actual concentration should be used. Besides, the actual and the detected values do not differ much. The concentration of the ethyl vanillin (as in Table 2.7a) is not constant, as each concentration is obtained from dilution from the previous stock solution.

2.2.3.4 Silylation time and temperature

Previous authors had used different combination of silylation temperatures and times: 50^0C for 1 hour (Hedges and Parker, 1976), 60^0C for 10 minutes (Hedges and Ertel, 1982), 90^0C for 30 minutes (Wilson *et al.*, 1985), 40^0C for 2 hours (Readman *et al.*, 1986) and at room temperature for 1 hour (Miltner and Emeis, 2001). In order to determine the best silylation conditions, five sets of experiments (in duplicate) with different silylating conditions, were carried out: 60^0C for 10 minutes, 60^0C for 30 minutes, 90^0C for 10 minutes, 90^0C for 30 minutes and 40^0C for 2 hours. Hence the 60^0C and 90^0C for 10 and 30 minutes, and 40^0C for 2 hours were tested here. The most suitable condition is chosen based on the smallest difference between the actual and detected concentration (Table 2.8a).

Table 2.8 Silylation conditions.

(a) The actual and detected lignin phenol concentrations, and the differences between the two concentrations due to different silylation conditions.

	Lignin	Actual Conc. (mg/g)	Detected concentrations (mg/g)				Difference between actual and detected concentrations			
			60°C, 10min	60°C, 10min	60°C, 30min	60°C, 30min	60°C, 10min	60°C, 10min	60°C, 30min	60°C, 30min
1	p-Hb	9.66	11.21	11.17	11.24	11.05	1.55	1.51	1.58	1.39
2	p-Ha	11.17	13.38	13.36	13.46	14.01	2.21	2.19	2.29	2.84
3	Vanillin	11.21	11.34	11.33	9.52	12.28	0.13	0.12	-1.69	1.07
4	EV	9.61	9.61	9.61	9.61	9.61	0	0	0	0
5	Acetovan	10.63	11.57	11.39	9.17	9.19	0.94	0.76	-1.46	-1.44
6	p-Hba	9.03	12.72	12.78	12.51	14.43	3.69	3.75	3.48	5.4
7	Syringal	8.8	5.59	7.74	7.28	8.73	-3.21	-1.06	-1.52	-0.07
8	Acetosyr	9.88	11.04	8.81	12.76	17.22	1.16	-1.07	2.88	7.34
9	Va	11.83	10.11	14.13	8.74	9.11	-1.72	2.3	-3.09	-2.72
10	Sa	9.43	9.8	9.66	9.6	11.9	0.37	0.23	0.17	2.47
11	p-cou	9.02	11.42	11.13	11.02	13.89	2.4	2.11	2	4.87
12	Fe	10.28	7.75	8.3	9.35	12.4	-2.53	-1.98	-0.93	2.12

		Actual conc. (mg/g)	Detected concentrations (mg/g)				Difference between actual and detected concentrations			
			90°C, 10min	90°C, 10min	90°C, 30min	90°C, 30min	90°C, 10min	90°C, 10min	90°C, 30min	90°C, 30min
1	p-Hb	9.66	11.08	11.14	11.19	11.13	1.42	1.48	1.53	1.47
2	p-Ha	11.17	13.28	13.33	13.39	13.39	2.11	2.16	2.22	2.22
3	Vanillin	11.21	11.27	11.33	11.34	11.36	0.06	0.12	0.13	0.15
4	EV	9.61	9.61	9.61	9.61	9.61	0	0	0	0
5	Acetovan	10.63	11.26	11.28	11.26	11.31	0.63	0.65	0.63	0.68
6	p-Hba	9.03	12.61	12.71	12.75	12.8	3.58	3.68	3.72	3.77
7	Syringal	8.8	7.28	7.28	7.28	7.3	-1.52	-1.52	-1.52	-1.5
8	Acetosyr	9.88	8.77	8.74	8.78	8.8	-1.11	-1.14	-1.1	-1.08
9	Va	11.83	13.95	13.98	14.06	14.12	2.12	2.15	2.23	2.29
10	Sa	9.43	9.51	9.55	9.57	9.71	0.08	0.12	0.14	0.28
11	p-Cou	9.02	11.6	11.68	11.65	11.7	2.58	2.66	2.63	2.68
12	Fe	10.28	10.31	10.34	10.25	10.16	0.03	0.06	-0.03	-0.12

		Actual conc. (mg/g)	Detected concentrations (mg/g)		Difference between actual and detected concentrations	
			40°C, 2 hrs	40°C, 2 hrs	40°C, 2 hrs	40°C ,2 hrs
1	p-Hb	9.66	11.16	11.2	1.5	1.54
2	p-Ha	11.17	13.33	13.4	2.16	2.23
3	Vanillin	11.21	11.38	11.38	0.17	0.17
4	EV	9.61	9.61	9.61	0	0
5	Acetovan	10.63	11.22	11.27	0.59	0.64
6	p-hba	9.03	12.7	12.75	3.67	3.72
7	Syringal	8.8	7.29	7.33	-1.51	-1.47
8	Acetosyr	9.88	8.77	8.81	-1.11	-1.07
9	Va	11.83	14.01	14.05	2.18	2.22
10	Sa	9.43	9.59	9.61	0.16	0.18
11	p-Cou	9.02	11.66	11.74	2.64	2.72
12	Fe	10.28	10.06	10.23	-0.22	-0.05

Conc = concentration

(b) Silylation conditions, the mean difference between the actual and detected lignin phenol concentrations.

No	Lignin phenol standards	Mean difference between the actual and detected concentrations with different time and temperature.				
		60°C, 10 mins	60°C, 30 mins	90°C, 10 mins	90°C, 30 mins	40°C, 2 hrs
1	p-Hb	1.53	1.49	1.45*	1.5	1.52
2	p-Ha	2.2	2.57	2.135*	2.22	2.20
3	Vanillin	0.125	-0.31	0.09*	0.14	0.17
4	EV	0	0	0	0	0
5	Acetovan	0.85	-1.45	0.64	0.66	0.62*
6	p-hba	3.72	4.44	3.63*	3.75	3.7
7	Syringal	-2.14	-0.795*	-1.52	-1.51	-1.49
8	Acetosyr	0.045*	5.11	-1.125	-1.09	-1.09
9	Va	0.29*	-2.91	2.135	2.26	2.2
10	Sa	0.3	1.32	0.1*	0.21	0.17
11	p-Cou	2.255	3.44	2.62*	2.66	2.68
12	Fe	-2.255	0.60	0.045*	-0.075	-0.135

* indicates the smallest difference among all the different temperature and time sets

From data analysis using single factor ANOVA, there were no significant differences ($p < 0.05$) between silylation results due to 60°C 10 minutes and 60°C 30 minutes, between 90°C 10 minutes and 90°C 30 minutes, nor between 90°C for 10 minutes and 40°C for 2 hours. Hence the duration of reaction did not have a significant effect on the experimental outcome. 90°C 10min was used because this combination gave the smallest differences (Table 2.8b) among all other conditions although these differences are not significant. Also the shorter and more time-saving duration was chosen.

2.2.3.5 CuO oxidation conditions

Different authors had subjected the CuO oxidation to different conditions (Table 1.1). Cupric oxide oxidation of a lignin hydrolytic was subjected to different temperatures and times. Results are given in Table 2.9a and the mean values given in Table 2.9b. Lignin hydrolytic is a lignin powder produced from plant materials, manufactured by Sigma Aldrich. Four sets of

experiments were carried out: 170°C for 2 hours, 170°C for 3 hours, 155°C for 2 hours, and 155°C for 3 hours. The oxidation product after CuO oxidation at 170°C was so concentrated and dried that it was difficult to wash with NaOH solution into the centrifuge tubes hence the results obtained were not consistent (see results for 170°C in Table 2.9a). A lower temperature enabled most of the oxidation products to be retained and higher concentrations of lignin-derived phenols were detected (Table 2.9a). There was no significant difference (ANOVA: $p>0.05$) between CuO oxidation at 155°C for 2 and 3 hours. The 3 hours was chosen to enable more complete oxidation.

Table 2.9 CuO oxidation conditions.

(a) The actual results.

No	Lignin phenols	Concentrations of lignin phenol detected from different CuO oxidation programs (mg/g)							
		170°C, 2 hrs	170°C, 2 hrs	170°C, 3 hrs	170°C, 3 hrs	150°C, 2 hrs	150°C, 2 hrs	150°C, 3 hrs	150°C, 3 hrs
1	p-Hydroxybenzaldehyde	0.05	0.04	0.04	0.10	0.38	0.43	0.50	0.48
2	p-Hydroxyacetophenone	0.37	0.26	0.31	0.86	0.39	0.48	0.43	0.40
3	Vanillin	0.25	0.18	0.31	1.30	0.65	0.60	0.51	0.43
4	Ethyl vanillin	0.22	0.22	0.22	0.22	0.23	0.23	0.22	0.22
5	Acetovanillone	0.30	0.25	0.40	0.60	0.39	0.49	0.43	0.41
6	p-Hydroxybenzoic acid	1.38	1.62	5.05	16.1	0.45	0.56	0.55	0.53
7	Syringaldehyde	0.30	0.17	0.03	0.06	0.84	0.60	1.57	1.50
8	Acetosyringone	0.35	0.29	0.49	0.38	0.67	0.84	0.64	0.73
9	Vanillic acid	1.62	1.39	4.02	9.98	0.58	0.72	0.89	0.88
10	Syringic acid	2.26	1.93	5.22	9.48	0.99	1.16	1.01	1.23
11	p-Coumaric acid	6.64	5.15	4.38	0.81	9.20	12.76	6.48	7.62
12	Ferulic acid	0.88	0.68	0.70	0.35	1.44	1.98	1.00	1.40

The results in the shaded column are not used in the calculation of the mean values in Table 2.8b as these results differ much from the first three columns.

(b) Mean lignin phenol concentrations for different time and temperature sets.

No	Lignin phenols	Mean lignin phenol concentration (mg/g)			
		170°C, 2hrs	170°C, 3hrs	155°C, 2 hrs	155°C, 3hrs
1	p-Hydroxybenzaldehyde	0.05	0.04	0.41**	0.49*
2	p-Hydroxyacetophenone	0.32	0.31	0.44*	0.42**
3	Vanillin	0.22	0.31	0.63*	0.47**
4	Ethyl vanillin	0.22	0.22	0.23	0.22
5	Acetovanillone	0.28	0.4	0.44*	0.42**
6	p-Hydroxybenzoic acid	1.50**	5.05*	0.51	0.54
7	Syringaldehyde	0.24	0.03	0.72**	1.54*
8	Acetosyringone	0.32	0.49	0.76*	0.69**
9	Vanillic acid	1.51**	4.02*	0.65	0.89
10	Syringic acid	2.10**	5.22*	1.08	1.12
11	p-Coumaric acid	5.90	4.38	10.98*	7.05**
12	Ferulic acid	0.78	0.7	1.71*	1.20**

* shows the highest concentration obtained for the particular lignin phenol after treatment with the same temperature. ** shows the second highest concentrations obtained.

2.2.3.6 Lignin elucidation

Lignin hydrolytic powder (Sigma Aldrich) was subjected to the CuO oxidation process. The purpose was to confirm the ability of the CuO oxidation method to elucidate and quantify lignin phenols. Analysis of approximately 0.053g (53mg) lignin hydrolytic powder yielded total lignin of 33.24 mg/g, S = 6.96 mg/g, V = 4.48 mg/g, and C = 21.80 mg/g. Hence S/V=1.56, C/V=4.90 and (Ad/Al)v= 0.16. Complete results are given in Table 2.10 and the chromatogram shown in Figure 2.12.

From the results, it seems that lignin hydrolytic is composed mainly of cinnamyl phenols. This, as well as the high C/V ratio, indicates that this lignin phenol was most probably manufactured from non-woody plant tissues. Stable carbon isotopic analysis of the lignin hydrolytic (section 3.5.1) yielded $\delta^{13}C$ of $-14.28 \pm 0.25‰$, which is more closely related to $\delta^{13}C$ values of C4 plants, as Gordon and Goni (2003) found that C4 plants are more isotopically enriched with an average $\delta^{13}C$ value of $-13‰$.

Table 2.10 CuO oxidation of lignin hydrolytic.

No.	Lignin phenol	Lignin phenol concentration (mg/g sample)		
		1	2	Mean
1	p-Hb	1.4474	1.7581	1.6028
2	p-Ha	0.8228	0.9475	0.8851
3	Van	3.0538	3.5511	3.3025
4	Acetovan	0.6040	0.7149	0.6594
5	p-Hba	0.6365	0.7443	0.6904
6	Syringal	4.8211	5.3363	5.0787
7	Acetosyr	1.6850	1.9353	1.8101
8	Sa	0.0623	0.0742	0.0683
9	Va	0.3800	0.6594	0.5197
10	p-Cou	15.8798	15.7422	15.8135
11	Fe	5.5976	6.3718	5.9847
	S+V+C	32.0837	34.3901	33.2369
	S	6.5685	7.3458	6.9571
	V	4.0378	4.9254	4.4816
	C	21.4774	22.1189	21.7982
	S/V	1.6267	1.4914	1.5591
	C/V	5.3191	4.4908	4.9049
	(Ad/Al)v	0.1244	0.1857	0.1551

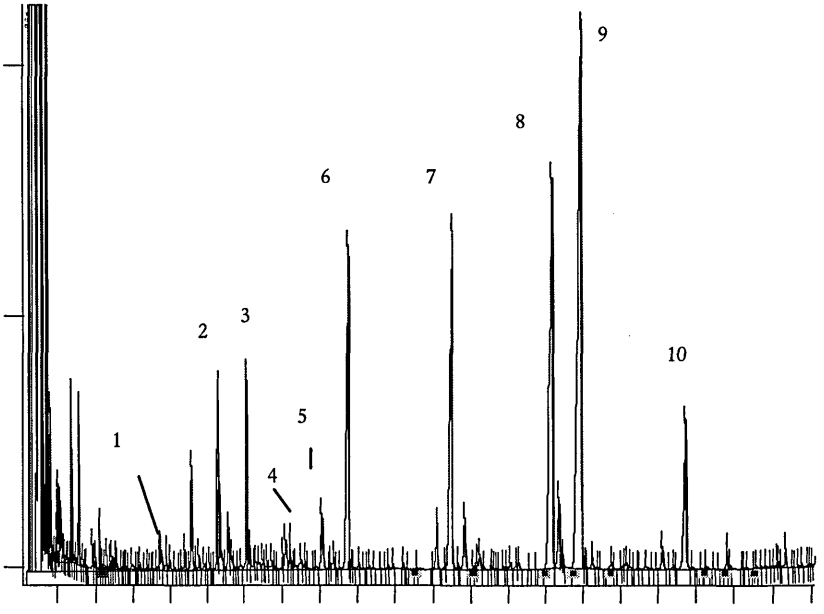


Figure 2.12 Chromatogram showing lignin phenols elucidated from lignin hydrolytic after CuO oxidation. Lignin phenols detected are: 1) p-hydroxybenzaldehyde, 2) p-hydroxyacetophenone, 3) vanillin, 4) ethyl vanillin, 5) acetovanillone, 6) p-hydroxybenzoic acid, 7) vanillic acid, 8) syringic acid, 9) p-coumaric acid and 10) ferulic acid.

2.2.3.7 Sample reproducibility

One sample RE6.1 (20.3.2001) 0-1cm was subjected to CuO oxidation five times, and another RE6.3 (20.2.2001)0-1cm was subjected to three times of analysis. Results are given in Table 2.11. Omitting the highest CV of 38.10%, the coefficient of variation (CV) ranges from 1.3 to 15.3% (Tables 2.11, a and b). Usually in our sampling three sediment cores were taken from one location. Only sediment from two of the cores will be subjected to CuO oxidation and the CV ranges for these duplicate results are within the range obtained here. In future duplicate results for each location are obtained.

Table 2.11 Reproducibility of sediment samples.

(a) Lignin parameters for RE6.1 (20.3.2001)0-1cm sediment.

No	Lignin phenol	Lignin phenol concentration (mg/g sediment)						
		1	2	3	4	5	Mean ±SD	CV (%)
1	p-Hb	0.22	0.29	0.22	0.19	0.23	0.23 ± 0.011	4.783
2	p-Ha	0.37	0.38	0.43	0.36	0.45	0.40 ± 0.015	3.750
3	Van	0.70	0.73	0.78	0.73	0.79	0.75 ± 0.014	1.867
4	Acetovan	0.17	0.15	0.17	0.22	0.34	0.21 ± 0.025	11.905
5	p-Hba	0.32	0.30	0.33	0.35	0.33	0.33 ± 0.005	1.515
6	Syringal	0.28	0.30	0.37	0.22	0.17	0.27 ± 0.026	9.630
7	Acetosyr	0.20	0.18	0.23	0.16	0.22	0.20 ± 0.010	5.000
8	Sa	0.40	0.36	0.37	0.40	0.37	0.38 ± 0.007	1.842
9	Va	0.41	0.37	0.39	0.39	0.41	0.39 ± 0.005	1.282
10	p-Cou	0.29	0.28	0.32	0.31	0.32	0.30 ± 0.007	2.333
11	Fe	0.32	0.34	0.37	0.33	0.29	0.33 ± 0.009	2.727
	S+V+C	2.76	2.71	3.00	2.76	2.91	2.83 ± 0.046	1.625
	S	0.87	0.84	0.97	0.78	0.77	0.84 ± 0.026	3.095
	V	1.28	1.25	1.34	1.34	1.54	1.35 ± 0.034	2.519
	C	0.61	0.62	0.69	0.65	0.61	0.63 ± 0.012	1.905
	S/V	0.68	0.67	0.72	0.58	0.50	0.63 ± 0.032	5.079
	C/V	0.47	0.49	0.52	0.48	0.39	0.47 ± 0.014	2.979
	(Ad/Al)v	0.58	0.50	0.51	0.54	0.52	0.53 ± 0.011	2.075

(b) Lignin parameters for RE6.3 (20.3.2001)0-1cm sediment.

No	Lignin phenol	Lignin phenol concentration (mg/g sediment)				
		1	2	3	Mean±SD	CV (%)
1	p-Hb	0.22	0.28	0.13	0.25 ± 0.021	38.10
2	p-Ha	0.36	0.26	0.30	0.31 ± 0.021	6.774
3	Van	0.60	0.47	0.42	0.50 ± 0.040	8.000
4	Acetovan	0.21	0.34	0.48	0.34 ± 0.052	15.294
5	p-Hba	0.31	0.33	0.44	0.36 ± 0.031	8.611
6	Syringal	-	0.07	0.08	0.07 ± 0.004	5.714
7	Acetosyr	0.17	0.19	0.15	0.17 ± 0.077	4.529
8	Sa	0.37	0.37	0.50	0.41 ± 0.033	7.976
9	Va	0.38	0.38	0.43	0.40 ± 0.014	3.500
10	p-Cou	0.25	0.34	0.31	0.30 ± 0.019	6.333
11	Fe	0.29	0.34	0.28	0.30 ± 0.014	4.500
	S+V+C	2.28	2.51	2.65	2.48 ± 0.077	3.097
	S	0.53	0.64	0.72	0.63 ± 0.039	6.111
	V	1.20	1.19	1.34	1.24 ± 0.037	2.944
	C	0.55	0.68	0.59	0.61 ± 0.029	4.738
	S/V	0.45	0.54	0.54	0.51 ± 0.023	4.529
	C/V	0.46	0.57	0.44	0.49 ± 0.031	6.286
	(Ad/Al)v	0.64	0.80	1.03	0.82 ± 0.079	9.622

SD = standard deviation; CV = coefficient of variation

2.2.3.8 Lignin in D/POM

Water samples were collected from River Creran as detection of lignin in the dissolved (<0.45μm) and particulate fractions (>0.45μm) indicates that lignin input originates from the river and was transported into the loch. Water samples were collected before and after winter as there was usually decrease in the summer freshwater runoff into the loch, and increase due to the winter discharge resulting in large input of dissolved and particulate components (Asvall, 1976; Holtan, 1976; Tollan, 1976).

Water samples from River Creran were filtered with 'Whatman' 25mm diameter GF/F glass microfibre filters. Before filtration, the filters were ashed (500°C overnight) to eliminate any residual organic matter (Cauwet and Mackenzie, 1993). Dry filters were cooled and weighed. 2207ml of water samples were then filtered under vacuum, for a few hours. Filters were then

heated in an oven at 60°C for about 2 hours until total dryness, and then weighed again to obtain the dry weight of the particulate material. The filtrates were kept frozen until analysis. The filtrates were then subjected to the CuO oxidation process in order to determine lignin concentration in the particulate fraction. Filtered water was evaporated to near dryness and subjected to the CuO oxidation to determine lignin in the dissolved fraction. The CuO oxidation process for the filtrates and filtered water was the same as the CuO oxidation process for the sediment samples; the only difference being the sediment sample is replaced with the filtrates and filtered water, respectively (Figure 2.8).

Method Validation

Method validation was carried out to determine whether the pre-combusted filters introduced any new and interfering peaks to the chromatograms. Figure 2.13 of a chromatogram shows the presence of only the internal standard ethyl vanillin, hence filters do not introduced any interfering peaks.

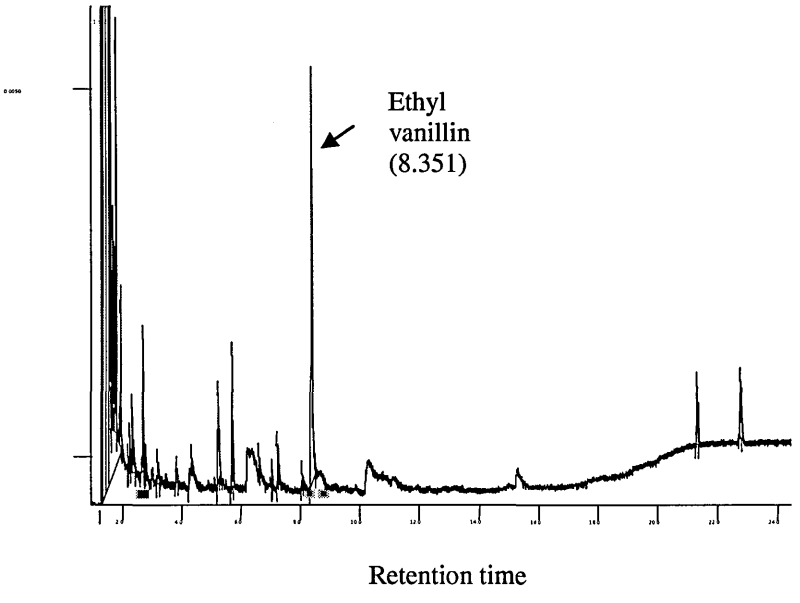


Figure 2.13 Chromatogram for CuO oxidation of precombusted filters. CuO oxidation of precombusted filters results in only the internal standard ethyl vanillin, its retention time which is 8.351.

2.2.3.9 Calculation of lignin phenol concentration

Calculation of lignin phenol concentration was based on the ratio of its peak area and the peak area of the internal standard ethyl vanillin, and is obtained from Braithwaite and Smith (1965).

Calculation of Response Factor (RF)

The size of a spectral peak is proportional to the amount of the substance that reaches the GC detector. No detector responds equally to different compounds. The elution of a component from the GC, its retention time, its elution volume, and hence its detected concentration, is affected by other components in the GC. Hence the original and detected concentrations are not the same. The response factors (RF) for each lignin phenols are calculated to cancel out any variability. The concentration of a lignin phenol was then corrected to this ratio. RF value for each lignin phenol does not deviate much each time a new standard mixture was used. The calculation of RF is given here.

In a standard mixture:

The individual component with concentration C_x has a peak area A_x. Usually the concentration used was approximately 0.5mg/ml.

Normalizing to 1mg: $A_x/C_x = A_C$

For the internal standard (IS) with a peak area Ay and concentration C_y:

Normalizing to 1mg: $A_y/C_y = A_{IS}$

Response factor for the component $= A_C/A_{IS} = R_C$

Example of a calculation of RF for a component in a standard mixture (Table 2.11):

Peak area of p-hydroxybenzaldehyde (p-hb) is 99185

Normalizing to 1mg, peak area = 99185/0.574 = 172 796.16

Peak area of the internal standard ethyl vanillin (EV) is 104273

Normalizing to 1mg, peak area = 104273/0.628 = 166039.8

Response factor for p-hb = 172796.16/166039.8 = 1.04

The RF values for all individual lignin phenols are then calculated using this method and results are given in Table 2.12.

Table 2.12 Calculation of RF.

No	Lignin	Original concentration (mg/ml)	Peak area (as in the chromatogram)	RF
1	p-Hydroxybenzaldehyde	0.574	99185	1.04
2	p-Hydroxyacetophenone	0.906	170299	1.13
3	Vanillin	0.860	143930	1.01
4	Ethyl vanillin *	0.628	104273	1.00
5	Acetovanillone	0.727	134908	1.12
6	p-hydroxybenzoic acid	0.564	127294	1.36
7	Syringaldehyde	0.766	108615	0.85
8	Acetosyringone	0.610	77084	0.76
9	Syringic acid	0.569	205357	2.17
10	Vanillic acid	1.025	95917	0.56
11	p-Coumaric acid	1.024	215807	1.27
12	Ferulic acid	0.520	92361	1.07

* Ethyl vanillin is the internal standard.

Calculation of lignin phenol concentration

The peak area is proportional to the amount of the eluted component and also depends on the response factor. Therefore, for the peak area (A) and concentration (C), A = R x C, where R = response factor, RF. Calculation of the concentration of a component is derived here.

For an individual component: $A_C = R_C \times C_C$

For the internal standard: $A_{IS} = R_{IS} \times C_{IS}$

$A_C/A_{IS} = (R_C \times C_C)/(R_{IS} \times C_{IS})$

The relative response of a component (R_R) to the internal standard is therefore:

$$R_R = A_C/A_{IS} = (R_C \times C_C)(R_{IS} \times C_{IS})$$

From the above equation, the concentration of each component is derived:

$$C_C = (A_C \times R_{IS} \times C_{IS})/(A_{IS} \times R_C)$$

$$= (A_C \times C_{IS})/(A_{IS} \times R_C), \qquad (\text{as } R_{IS} = 1)$$

Example of a calculation of the concentration of a component is given here:

Concentration of the internal standard ethyl vanillin (EV) is 5mg/10ml diethyl ether, or 0.5mg/ml diethyl ether. 100µl from this standard solution is spiked to the sample after the CuO oxidation at 550°C for 3h. Hence EV spiked into the sample = 0.05mg. From the chromatogram of a sediment sample, the peak area for p-hydroxybenzaldehyde (p-hb) is 339, and peak area of ethyl vanillin is 8993.

Hence, p-hb = $(339 \times 0.05)/(8993 \times R_c)$

Approximately 0.5g sediment sample was used and R_c=1.04 (from Table 2.12),

Concentration of p-hb per g of sediment = $(339 \times 0.05)/(8993 \times 1.04) \times 1/0.5$

$= 0.003$ mg/g

2.2.3.10 Comparison between present and previous validation experiments

The validation experiments carried out for lignin analysis in this study (Section 2.2.3) is then compared with validation experiments carried out in the past, the classic paper for such studies being that by Hedges and Ertel (1982). Some distinctive features are given below.

1. Hedges and Ertel (1982) had carried out the CuO oxidation in 10ml Monel 'minibombs' sealed with Teflon-lined screw caps, four such minibombs were loaded into a 200ml Parr bomb allowing oxidation up to four samples at once. The CuO oxidation was carried out at 170°C on a platform shaker. The 200ml outer bomb was heated in a snug brass cylinder that was wrapped with electrical heating tape and asbestos insulating cloth inside an Al tape covering. The reaction temperature was maintained with a temperature controller fitted with an armoured platinum temperature probe mounted in an insulated packet in contact with the brass heating sleeve.

   In this research, the CuO oxidation was carried out in 'minibombs' sealed with Teflon-lined screw caps. These were not loaded into another 200ml Parr bomb, and the CuO oxidation was not carried out on a platform shaker. In the contrary, the CuO oxidation was carried out in 'minibombs' which were heated inside a used GC oven. A thermometer was put inside the oven to make sure the temperature was correct. The temperature was set to rise from room temperature to 155°C in 20 minutes. A lower temperature was used in order to prevent superoxidation due to increased temperature. Besides, from the validation experiments in Section 2.2.3.5, it was found that the lower temperatures give better lignin phenol yields.

   In this work, there were no facilities to ensure the conditions of the outside and inside of the minibombs the same, and no platform shaker. It was sufficient to shake the bombs manually once every one hour for three hours. Reeves (1988) had similar findings. Besides, superoxidation could not have occurred as discussed in Point 4.

2. In Hedges and Ertel (1982), after introduction of a radioactive tracer each minibomb was charged with 1g of CH_2Cl-extracted CuO powder, 25-100mg ammonium ferrous sulphate $Fe(NH_4)_2(SO_4)_2.6H_2O$ which was used as oxygen scavenger, 7.0ml of 8% (wt/wt) aqueous NaOH, and a small stainless steel ball agitator.

   In this research, the stainless steel agitators were not added to the minibombs as it was sufficient to shake the contents of the bombs manually (Reeves, 1988). Reeves (1988) also found that a thick iron hydroxide precipitate was formed when ammonia ferrous sulphate was heated with sodium hydroxide, as a result ammonia ferrous sulphate was omitted from this step.

3. Hedges and Ertel (1982) added the internal standard ethyl vanillin together with pyridine and the silylating reagent, that is, the standard was added after the extraction process. Readman *et al.* (1986) added the ethyl vanillin after the extraction process and before the product was being evaporated to dryness. In this work the ethyl vanillin was added just before the extraction process with diethyl ether so that it was extracted three times with diethyl ether along with the sample. Of the three methods, the lowest recovery of ethyl vanillin would be from the third method, as the ethyl vanillin was added earliest during the CuO oxidation procedure. This will result in relatively higher concentrations of other lignin phenols.

   In order to study the recovery of the lignin phenols due to its addition at different oxidation stages, several experiments were carried out. The lignin phenols standard was added before the CuO oxidation process, and before and after the extraction process, and the results are given in Table 2.13. It is observed that the later the standard is added during the process, the higher the lignin concentrations are recovered, hence the less difference between the original and detected concentrations.

In reality, the addition of ethyl vanillin just before the extraction process will result in more loss of the standard, hence relative higher concentrations of other lignin phenols will be detected. This will cancel out the lost of the lignin phenols during the process.

Table 2.13 The recovery of lignin phenols due to addition of ethyl vanillin at different stages.

Lignin	Original conc (mg/g)	Before CuO (mg/g)				Before extraction (mg/g)				After extraction (mg/g)			
		1	2	mean	Dif	1	2	mean	Dif	1	2	mean	Dif
4-Hb	0.981	0.68		0.68	0.30	0.77	0.62	0.70	0.29	0.82	0.80	0.81	0.17
4-Ha	1.073	0.40	0.66	0.53	0.54	0.94	0.74	0.84	0.23	0.97	0.94	0.96	0.12
Vanillin	1.041	0.38	0.63	0.51	0.54	0.67	0.55	0.61	0.43	0.73	0.72	0.73	0.32
EV	1.025	1.03	1.03	1.03	-0.01	1.03	1.03	1.03	-0.01	1.03	1.03	1.03	-0.01
Acetovan	1.067	0.34	0.57	0.46	0.61	0.78	0.58	0.68	0.39	0.84	0.80	0.82	0.25
4-Hba		2.48	5.09	3.79	-3.79	1.10	0.86	0.98	-0.98	1.07	1.03	1.05	-1.05
Syring					0.00	0.29	0.22	0.26	-0.26	0.57	0.56	0.57	-0.57
Sa	1.033	0.13	0.62	0.38	0.66	0.17	0.11	0.14	0.89	0.80	0.78	0.79	0.24
p-Cou	0.975	0.30	0.69	0.50	0.48	0.82	0.53	0.68	0.30	0.95	0.91	0.93	0.05
Fe	0.954	0.08	0.12	0.10	0.85	0.07	0.04	0.06	0.90	0.71	0.67	0.69	0.26

Dif = difference between the original and mean concentrations.

4. Some of the precautions taken in this experiment are as follows:

- All the diethyl ether was freshly treated with ammonium ferrous sulphate to remove peroxides, as syringaldehyde is particularly sensitive to peroxides and the percentage losses increase with decreasing sample size (Hedges and Ertel, 1982). In this work, the percentage weight losses of the syringaldehyde were not tested, but the ammonium ferrous sulphate treatment was followed.

- Immediate analysis of the oxidation product after silylation process prevents the carbonyl losses which are more pronounced for the aldehydes than ketone. Geometric isomerization of the cinnamyl phenols in the silylated standard and sample solutions could also occurred. Ferulic acid also isomerizes faster than p-coumaric acid. However, geometric isomerization is too slow to have a measurable effect when the GC analysis is performed immediately after silylation (Hedges and Ertel, 1982). In this work, these

were also not monitored, but the precaution taken was immediate analysis of the silylated sample.

- Superoxidation, due to introduction of oxygen into the minibombs or due to elevated reaction temperature, would result in consistently high acid/aldehyde ratios within the p-hydroxyl, vanillyl and syringyl phenol families [abbreviated as (Ad/Al)p, (Ad/Al)v and (Ad/Al)s, respectively], as these high ratios reflect conversion of aldehydes to the corresponding carboxylic acids (Hedges and Ertel, 1982). Hence, in this work: between 155°C and 170°C, the 155°C was chosen as the temperature for the CuO oxidation process.

  It was found that the (Ad/Al)v results for Lochs Creran and Etive are slightly higher than some other locations worldwide (see Section 4.2.1.3). Could this be due to superoxidation, resulting in higher carboxylic acids than aldehydes? Several reasons indicate otherwise.

  (i) One reason is that the (Ad/Al)v values from Lochs Creran and Etive were not distinctly higher than other reported values. These (Ad/Al)v values are within the range of the past values, only that they occurred near the upper limit. Besides Lochs Creran and Etive, the other locations with (Ad/Al)v values higher than '1' is the Gulf of Mexico (see Section 4.2.1.3 for explanations). Also these were reported in the mean values, the individual value for each location might be higher.

  (ii) Secondly, it was found that the vanillyl and syringyl groups yielded more aldehyde than the acid and ketone groups (see Section 3.2.1.1, Table 3.2). If superoxidation occurred, the acid group would be found in higher concentration.

  (iii) The syringic acid/syringaldehyde ratio, (Ad/Al)s and the p-hydroxybenzoic acid/p-hydroxybenzaldehyde ratios are also investigated. Hedges *et al.* (1982) found that the (Ad/Al)v, (Ad/Al)s and (Ad/Al)p for Lake Washington sediments were 0.43, 0.31 and 1.02 respectively. Requejo *et al.* (1986) found that for the Narragansett Bay sediments,

the (Ad/Al)v, (Ad/Al)s and (Ad/Al)p ranged 0.24-0.84, 0.39-5.08, and 0.23-5.09. Gough *et al.* (1993) found that the (Ad/Al)v and (Ad/Al)s values for these locations are as follow: River Rhone and delta (0.31-0.43 and 0.30-0.46), North West Mediterranean Basin (0.37-1.1 and 0.69-1.14), North East Atlantic (0.37-2.92 and 'non-detected' to 2.44). The (Ad/Al)v, (Ad/Al)s and (Ad/Al)p values for Lochs Creran and Etive (Section 3.2) are within these ranges.

- All glassware and apparatus are clean and dust free. These are soaked in 5% Decon for overnight, rinsed three times with distilled water and dried at 50°C in an oven (Reeves and Preston, 1989).

2.2.3.11 Lignin parameters

Some lignin parameters used in this thesis are as followed:

1) Total lignin in mg/g; calculation given in Section 2.2.3.9.
2) Λ (mg/100mg OC); calculation given in Section 4.2.
3) S/V and C/V ratios:

The relationships among these lignin groups are given by these equations:

$$S/V = \frac{\text{Syringaldehyde + acetosyringone + syringic acid}}{\text{Vanillin + acetovanillone + vanillic acid}}$$

$$C/V = \frac{\text{p-coumarinc acid + ferulic acid}}{\text{Vanillin + acetovanillone + vanillic acid}}$$

4) (Ad/Al)v, is the ratio of vanillic acid to vanillin.

2.2.4 Oxygen uptake from intact sediment core

Three sediment cores were collected from each location using Perspex core tubes (5.9cm i.d. x ca. 24cm long) and Craib corer (Figure 2.5). Seawater samples for the incubation of these cores were collected from LC1 at the depth of 10m below the surface water (Figure 2.14), as this depth was just high enough from the surface sediments to prevent materials re-suspension into the traps (also see Section 2.2.1.2 for other reason). Upon arrival at the laboratory, a container was filled with the seawater. This was incubated in a cold room at a constant temperature of about 10°C, as the average *in situ* bottom water temperature ranges 7°C-14°C, in order to reduce any disturbance due to sampling and subsequent transport. Sediment cores were held immersed in the water overnight, each core being bubbled with air at the surface layer so that oxygen concentration for the cores remain constant. The next morning, the whole container was aerated for half an hour. Submersible stirrers were then fitted onto the core tubes and were allowed to run throughout the experiment. The cores were stirred slowly to prevent stagnation of the overlying water, and yet, not to cause re-suspension of sediment. Oxygen uptake rates were determined from the decrease in dissolved oxygen in the overlying water from the intact incubated sediment cores (Parkes and Buckingham, 1986; Glud *et al.*, 1994; Overnell *et al.*, 1995b).

Figure 2.14 Water sample taken for the oxygen uptake test.

At time zero, just after the cores were sealed, replicates of three to five water samples were collected randomly from the tank using 10ml glass syringes. 24 hours after sealing, triplicate samples of the overlying water in each core tube were collected. The height of water column between the sediment surface and stirrer was measured.

Standardization of thiosulphate

The reagents used in the standardization procedures were as follow:

1. Alkaline iodide 40g NaOH

   Sodium iodide 90g NaI

   56ml H_2O → 100ml

2. Stock sodium thiosulphate $Na_2S_2O_3.5H_2O$ (MW=248)

   ≈ 7.44g + 1 pellet NaOH → 100m (= ≈ 0.3M = 0.15N)

3. Stock potassium iodate KIO_3 (MW=214)

   0.089175g (dried 105°C for 1 hour) → 100ml (= 0.0042M = 0.0125N)

4. H_2SO_4 (98% = 18.4M)

   34ml conc. → 100ml (= 6.25M = 12.5N)

Normality of iodate used was calculated as follows:

Weight of iodate = 0.107g

Molecular weight of KIO_3 = 214

Volume of standard = 100ml

$$\text{Normality of iodate} = \frac{0.107}{214} \times \frac{1000}{100} \times 3 = 0.015\text{N}$$

Diluted 10 times = 0.0015N

5ml of 0.0015N KIO_3 was fixed with 0.1ml alkaline iodide and 0.1ml H_2SO_4, followed by titration with 0.0015N thiosulphate. Potassium iodate reacts with potassium iodide in acid solution to liberate iodine. Reactions occur are as follow:

$$KIO_3 + 5KI + 3H_2SO_4 \longrightarrow 3K_2SO_4 + 3I_2 + 3H_2O$$

$$\Rightarrow \quad \cancel{K^+} + IO_3^- + 5\cancel{K^+} + 5I^- + 6H^+ + 3\cancel{SO_4^{2-}} \longrightarrow 6\cancel{K^+} + 3\cancel{SO_4^{2-}} + 3I_2 + 3H_2O$$

$$\Rightarrow \quad IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

This result is a yellow iodine solution. This solution is then titrated with approximately 0.0015N thiosulphate. The endpoint of the titration occurs when all the I_2 was reacted with the thiosulphate. Thiosulphate ion is a moderately strong reducing agent; with iodine, this ion is oxidized to tetrathiosulphate, the half reaction being (Skoog *et at.*, 1996; Hansen, 1999):

$$2S_2O_3^{2-} \longrightarrow S_4O_6^{2-} + 2e^-$$

and $\quad I_2 + 2e^- \longrightarrow 2I^-$

$\therefore \quad 2S_2O_3^{2-} + I_2 \longrightarrow S_4O_6^{2-} + 2I^-$

or $\quad 2Na_2S_2O_3 + I_2 \longrightarrow Na_2S_4O_6 + 2NaI$

$$\Rightarrow \quad \cancel{4Na^+} + 2S_2O_3^- \longrightarrow \cancel{2Na^+} + S_4O_6^{2-} + \cancel{2Na^+} + 2I^-$$

similarly, $\quad 2S_2O_3^{2-} + I_2 \longrightarrow S_4O_6^{2-} + 2I^-$

This parameter allowed the normality of the thiosulphate solution to be determined exactly.

Titration of samples

10ml of the water samples were collected in 10ml-glass syringes. Every 10ml sample was fixed with 0.1ml alkaline iodide and 0.1ml $MnSO_4$. The oxygen present in the sample will react with the manganese (II) hydroxide, $Mn(OH)_2$ to form manganese (III) hydroxide, $Mn(OH)_3$. $Mn(OH)_3$ then reacts with the I^- from alkaline iodide to form iodine.

$$2Mn(OH)_2 + \tfrac{1}{2} O_2 + H_2O \longrightarrow 2Mn(OH)_3$$

$$2Mn(OH)_3 + 2I^- + 6H^+ \longrightarrow 2Mn^{2+} + I_2 + 6H_2O$$

When titrated with thiosulphate, the thisulphate will react with I_2 to liberate I^-.

$$I_2 + S_2O_3^{2-} \longrightarrow 2I^-$$

Calculations:

$$\text{Thiosulphate normality} = \frac{\text{Iodate normality x [iodate]}}{\text{[Thiosulphate]}} \quad \text{N}$$

$$I_2 \text{ concentration} = \frac{\text{Thiosulphate normality x Thiosulphate volume (ml)}}{\text{Sample volume (ml)}} \quad \text{N}$$

$$O_2 \text{ concentration } (\mu M) = \frac{I_2 \text{ equilibrium concentration x } 1000000}{2}$$

$$\text{Flux (mmole/m}^2\text{/day)} = \frac{[O_2 \text{ conc. at 0 hour} - O_2 \text{ conc. (M)}] \text{ x overlying water height x } 24}{100 \text{ x time}}$$

Samples reproducibilities

The range of samples reproducibilities from triplicate analyses of individual cores (**Appendix 3**) is 0.10-7.90% coefficient of variation.

2.2.5 Loss on ignition

Method

Loss on ignition (LOI) was carried out in a temperature monitored muffle furnace. Approximately 0.5g of dried, ground and sieved sediment sample was weighed precisely into a crucible. Crucibles with sediment were then ashed (250°C for 16 hours). When cooled, the crucibles were reweighed. Sediments were then heated to 500°C (Kristensen and Andersen, 1987) for 16 hours (Sutherland, 1998). When cool, they were weighed again.

According to Kristensen (1990), the weight loss in the lower temperature range (130-280°C) was due to evaporation (i.e. dehydrogenation of hydroxylated aliphatic structures, decarboxylation of acid groups and generation of low molecular weight volatile compounds); oxidative degradative degradation of aliphatic carbohydrates; random chain-scission of weak bonds; cross linking and peroxide formation; formation of compounds of less ordered structure; cyclization; and formation of carbonaceous char. Plant materials rich in carbohydrates are combusted at the lower temperatures. The weight loss in the high temperature region (280-520°C) was due to oxidation of aromatic groups (polyphenolic compounds like lignin, humic substances and kerogens) and char. The percentages weight reduction after both 250°C and 500°C were measures of the sediment labile and refractory organic matter, respectively.

Mook and Hoskin (1982) found significant ($p<0.05$) weight losses between 200°C and 300°C, hence the mean 250°C is chosen to be used here. Kristensen and Andersen (1987) found that most calcite was combusted between 500-800°C, hence the 500°C is used in this study, as most

organic carbon would be completely separated with this temperature. Hirota and Szyper (1975; Section 1.5.4, Table 1.5) also separated organic and inorganic compounds by heating at 500°C.

Rp index was introduced by Kristensen (1990) to characterize the composition of various biogenic organic materials at different decomposition stages. It is defined as, Rp = PII/(PI + PII) where PI is the weight loss in the temperature range 0°C and 250°C and PII is the weight loss in the temperature range 250°C and 500°C. Calculations of percentage organic matter and Rp values are as follow:

Let, Ws= original weight of sediment sample (approximate 0.5g)

Wo = weight of crucible and sediment before combustion

W_{250} = weight of crucible and sediment after combustion at 250°C

W_{500} = weight of crucible and sediment after combustion at 500°C

PI = weight loss in temperature range 0°C and 250°C = $Wo - W_{250}$

PII = weight loss in temperature range 250°C and 500°C = $W_{250} - W_{500}$

and, Rp= PII / (PI + PII)

% labile OM = (PI/Ws) x 100%

% refractory OM = (PII/Ws) x 100%

$$\% \text{ total OM} = \frac{Wo - W_{500}}{Ws} \times 100\%$$

or, % total OM = % labile OM + % refractory OM

Method Validation

One sample was subjected to combustion at 250°C and 500°C for 15 times (Figure 2.15) and the results presented in Table 2.14. The mean, standard deviation and % probability for the % labile, % refractory, and % total organic matter (TOM) and the Rp values are then studied for each row and column (Table 2.15). Average % probability was in the range 3.17-38.53%. In future analysis, one LOI analysis was carried out for sediment from each core; hence triplicate analysis consists of three different cores. The % probability for the sediment samples were also within this range 10-20% so this is acceptable.

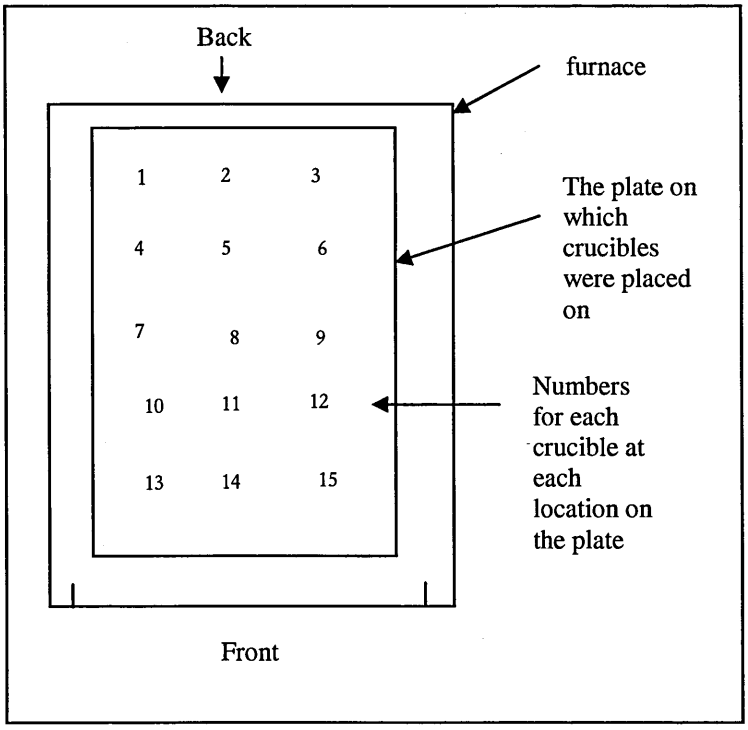


Figure 2.15 Positions of crucibles during the LOI validation experiments.

Table 2.14 Raw data for loss on ignition validation experiment.

Position no	Dry wt (g)	Crucible + sediment (g)			PI	PII	% labile OM	% refrac OM	% total OM	Rp
		Bf 250°C	Af 250°C	Af 500°C						
1	0.502	6.233	6.219	6.202	0.014	0.017	2.79	3.39	6.18	0.55
2	0.503	6.389	6.375	6.355	0.014	0.02	2.78	3.98	6.76	0.59
3	0.51	6.367	6.352	6.333	0.015	0.019	2.94	3.73	6.67	0.56
4	0.506	6.08	6.066	6.043	0.014	0.023	2.77	4.55	7.31	0.62
5	0.509	6.522	6.513	6.487	0.009	0.026	1.77	5.11	6.88	0.74
6	0.505	6.261	6.25	6.226	0.011	0.024	2.18	4.75	6.93	0.69
7	0.506	6.031	6.015	5.996	0.016	0.019	3.16	3.75	6.92	0.54
8	0.502	6	5.988	5.968	0.012	0.02	2.39	3.98	6.37	0.63
9	0.505	6.18	6.173	6.156	0.007	0.017	1.39	3.37	4.75	0.71
10	0.503	6.319	6.309	6.291	0.01	0.018	1.99	3.58	5.57	0.64
11	0.502	6.427	6.414	6.396	0.013	0.018	2.59	3.59	6.18	0.58
12	0.501	6.437	6.426	6.406	0.011	0.02	2.20	3.99	6.19	0.65
13	0.502	6.301	6.291	6.275	0.01	0.016	1.99	3.19	5.18	0.62
14	0.506	6.123	6.108	6.094	0.015	0.014	2.96	2.77	5.73	0.48
15	0.502	6.281	6.27	6.252	0.011	0.018	2.19	3.59	5.78	0.62

Bf = before, af = after, refrac = refractory, OM = organic matter, PI = the weight loss in the temperature range 0°C and 250°C and PII = the weight loss in the temperature range 250°C and 500°C. The position numbers are based on position in Figure 2.13.

Table 2.15 Loss on ignition reproducibility test. Positions of each crucible are designated in (parentheses). The average % probability for the % labile organic matter for the rows is 19.74%, for the column is 21.50% and overall average % probability is 20.40%. For the % refractory organic matter, the average coefficient of variation (CV) for the rows is 8.28% and for the column is 16.44% and overall average 11.34%. For the % total organic matter the average CV for the rows is 7.78%, average CV for the column is 11.90% and overall average CV is 9.93%. For the Rp values, the average CV for the rows is 9.42%, average CV for the column is 10.90% and overall average CV 9.98%.

(a) % labile organic matter.

					Mean±standard deviation	CV
	(1) 2.79	(2) 2.78	(3) 2.94	→	2.84 ±0.09	3.17
	(4) 2.77	(5) 1.77	(6) 2.18	→	2.24 ±0.50	22.32
	(7) 3.16	(8) 2.39	(9) 1.39	→	2.31 ±0.89	38.53
	(10) 1.99	(11) 2.59	(12) 2.20	→	2.26 ±0.30	13.27
	(13) 1.99	(14) 2.96	(15) 2.19	→	2.38 ±0.51	21.43
	↓	↓	↓			Ave=19.74%
Mean	2.54	2.50	2.18			
	±0.53	±0.46	±0.55			
CV	20.87	18.40	25.23	Ave=21.50%	Overall ave=20.40%	

(b) % refractory organic matter.

					Mean±standard deviation	CV
	(1) 3.39	(2) 3.98	(3) 3.73	→	3.70 ±0.30	8.11
	(4) 4.55	(5) 5.11	(6) 4.75	→	4.80 ±0.28	5.83
	(7) 3.75	(8) 3.98	(9) 3.37	→	3.70 ±0.31	8.38
	(10) 3.58	(11) 3.59	(12) 3.99	→	3.72 ±0.23	6.18
	(13) 3.19	(14) 2.77	(15) 3.59	→	3.18 ±0.41	12.89
	↓	↓	↓			Ave=8.28%
Mean	3.69	3.89	3.89			
	±0.52	±0.84	±0.53			
CV	14.09	21.59	13.62	Ave=16.43%	Overall ave=11.34%	

(c) % total organic matter.

					Mean±standard deviation	CV
	(1) 6.18	(2) 6.76	(3) 6.67	→	6.54 ±0.31	4.74
	(4) 7.31	(5) 6.88	(6) 6.93	→	7.04 ±0.24	3.41
	(7) 6.92	(8) 6.37	(9) 4.75	→	6.01 ±1.13	18.80
	(10) 5.57	(11) 6.18	(12) 6.19	→	5.98 ±0.36	6.02
	(13) 5.18	(14) 5.73	(15) 5.78	→	5.56 ±0.33	5.94
	↓	↓	↓			Ave=7.78%
Mean	6.23	6.38	6.06			
	±0.89	±0.46	±0.86			
CV	14.29	7.21	14.19	Ave=11.90%	Overall ave=9.93%	

(d) Rp values.

					Mean±standard deviation	CV
	(1) 0.55	(2) 0.59	(3) 0.56	→	0.57 ±0.02	3.51
	(4) 0.62	(5) 0.74	(6) 0.69	→	0.68 ±0.06	8.82
	(7) 0.54	(8) 0.63	(9) 0.71	→	0.63 ±0.09	14.29
	(10) 0.64	(11) 0.58	(12) 0.65	→	0.62 ±0.04	6.45
	(13) 0.62	(14) 0.48	(15) 0.62	→	0.57 ±0.08	14.04
	↓	↓	↓			Ave=9.42%
Mean	0.59	0.60	0.65			
	±0.05	±0.09	±0.06			
CV	8.47	15.00	9.23	Ave=10.90%	Overall ave=9.98%	

Samples reproducibilities

The range of samples reproducibilities from triplicate analyses of individual cores is 0.00-20.00% coefficient of variation. The CV of 38.53% was exceptional higher than others, so not included in this range.

2.2.6 CN analysis

The method for the sediment CN analysis was obtained from Verardo *et al.* (1990).

Organic carbon

Sediments were treated with sulfur dioxide solution 5% w/v (sulphurous acid) to remove the carbonate fraction. Approximately 10mg of sediment was precisely weighed into a 1.5ml plastic digestion vial and treated with 1ml sulphurous acid. The vials were then placed on a rack. In the fume cupboard, 1ml of sulphurous acid was dispensed into each vial using a syringe. If the sample contained carbonate material, it will effervesce and release carbon dioxide. The acid should be dispensed slowly so that the sediment would not froth over the side of the vial. It was also necessary to tap the side of the vial to release bubbles trapped at the bottom of the vial, as bubbles would prevent full contact between acid and the sample. Once the acid was applied, the samples were left in a desiccator for overnight. The vials and samples were then placed in a heater block at 70°C for 2 hours. The vials were then capped, and put into freezer for overnight. The next day, for drying purpose, the caps were removed and the vials with frozen sediments placed in the freeze-dryer for overnight. Tin capsules (8 x 5mm) were prepared by widening the mouth of an existing capsule, tapping the contents of a vial into the capsule, then compressing the capsule into a ball. This was ready to be analysed for carbon (c) and nitrogen (N) with a LECO CHN-900 analyser.

Total carbon

Approximately 10mg sediments non-treated with sulphurous acid were weighed. Sediments were weighed directly into the tin capsules and compressed, and ready to be analysed for C and N.

Calibration

Blanks (empty tin capsules) and a series of acetanilide standard weighing from 2.0 to 10.0mg were analyzed for C and N. Acetanilide has close approximate with the marine organic matter C:N ratio (Pocklington and Leonard, 1979). Molecular weight for acetanilide $C_6H_5.NH.CO.CH_3$ is 135.162. Percentages C and N in acetanilide are calculated:

$$\%\,C = (12.01 \times 8/135.162) \times 100\% = 71.09\%$$

$$\%\,N = (14.01/135.162) \times 100\% = 10.36\%$$

The %C and %N in different mass of acetanilide standard are then determined (Table 2.16) by multiplying the %C = 71.09% and %N = 10.36% with the mass. The gradient and intercept for %C and %N versus peak areas calibration curves (Figure 2.16) were then used to calculate the C and N contents in sediment samples:

$$\text{Mg of C} = \frac{\text{Peak height - Intercept}}{\text{gradient}}$$

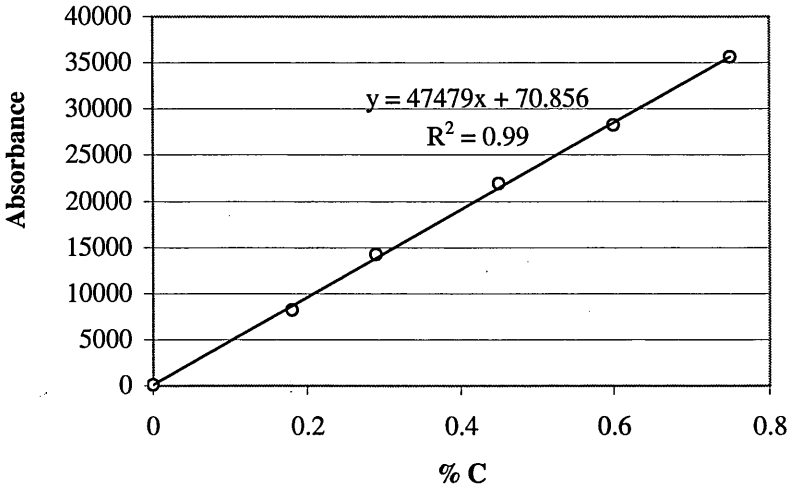
No standard reference material was used. In fact, a known amount of acetanilide was then subjected to the C and N analysis in order to determine whether this amount produced the correct amount of C and N.

Table 2.16 Acetanilide standard, the %C and %N.

Acetanilide standard (mg)	% C	% N
0.25	0.18	0.03
0.41	0.29	0.04
0.64	0.45	0.07
0.84	0.60	0.09
1.05	0.75	0.11

%C is calculated by multiplying the mg acetanilide with 71.09%. %N is obtained by multiplying mg acetanilide with 10.36%.

(a) Calibration curve for % C.



(b) Calibration curve for % N.

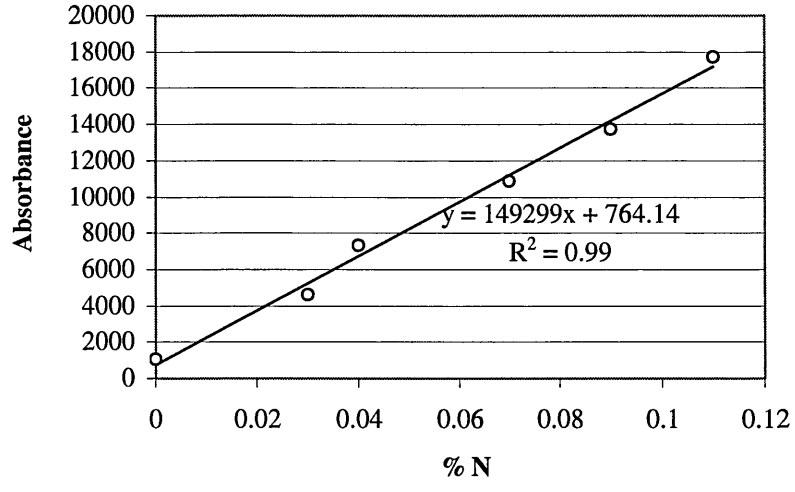


Figure 2.16 Calibration curves for CN analysis.

Samples reproducibility

Samples reproducibility for the %TC and %TN ranges 0-20.56% and 0-19.17% uncertainties.

2.2.7 Stable carbon isotopes

Approximately 0.1mg sediment was weighed into tin capsule, wrapped and ready for the stable carbon isotopes determination. The standard used was L-isoleucine, which was calibrated against a Pee Dee Belemnite (PDB) standard with $^{13}C/^{12}C$=88.99, which defines the zero per mil line on the δ-scale (Bashkin, 2002). Stable carbon isotopic ratios were determined using a "20-20 Stable Isotope Analyzer PD2 Europa Scientific Instrument". The $\delta^{13}C$ value is calculated from the measured carbon isotope ratios of the sample and standard gases (Degens, 1969; Boutton, 1991):

$$\delta^{13}C\ (‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 10^3$$

where $R_{sample} = {}^{13}C/{}^{12}C$ ratio in the sample, and $R_{standard} = {}^{13}C/{}^{12}C$ ratio in the standard.

Samples reproducibilities

Samples reproducibility for the $\delta^{13}C$ values range 0.03-16.87% uncertainties (see Table 3.14).

2.2.8 Phosphate analysis

The method used for phosphate analysis is obtained from Strickland and Parsons (1972), Aspila *et al.* (1976) and Koroleff (1976).

Inorganic phosphate

The freeze-dried and ground sediment was weighed accurately to 0.25g. This was washed with 20ml of 1M HCl into a 50ml centrifuge tube. Sediments were extracted with constant shaking of the centrifuge tubes for 16 hours at room temperature. The supernatant was then decanted. Sediment was washed again with 5ml HCl, centrifuged at 600g for 10 minutes, and the supernatant combined. This was ready to be analysed for phosphate.

Organic phosphate

The dry residue from the above procedure was transferred to a porcelain crucible and heated in a muffle furnace at 550°C for 2 hours. When cooled the sediment was transferred to a 50ml centrifuge tube, and extracted with 25ml 1N HCl for 16h at room temperature with constant shaking. This was then centrifuged at 600g for 10 minutes. The supernatant was then decanted and ready to be analysed for phosphate.

Working reagent

1. Ammonium molybdate solution: Dissolve 7.5g of analytical $(NH_4)_6Mo_7O_{24}.4H_2O$ in 250ml of distilled water. Store in plastic bottle out of direct sunlight, stable indefinitely.
2. Sulphuric acid solution: Add 70ml of concentrated acid (s.g. 182) to 450ml of distilled water. Store in glass or plastic bottle.
3. Ascorbic acid solution: Dissolve 13.5g L-ascorbic ($C_6H_8O_6$ = 176.13) acid in 250ml distilled water. Store in plastic bottle and keep in freezer.
4. Potassium antimony oxide tartrate solution: Dissolve 0.17g of reagent in 125ml of distilled water (this may be warmed). Store in glass or plastic bottle. Replace after several months.

The 'Working Reagent' was prepared by mixing 50ml of solution 1, 125ml of solution 2, 50ml of solution 3 and 25ml of solution 4. This was prepared daily before use.

Standard phosphate solution

Potassium dihydrogen orthophosphate (KH_2PO_4) was dried in the oven at 110°C and then put in the desiccator to cool. Exactly 136.1mg was dissolved in distilled water to which had been added 1ml 9M sulphuric acid and diluted to 100ml. This was stored in cold and was stable for months. Calculation of concentration of the PO_4^{3-} is as shown:

Molecular weight of KH_2PO_4 is 136.1. 136.1mg of KH_2PO_4 dissolved in 100ml of distilled water will give a molarity (M) of:

$$\text{Molarity of } KH_2PO_4 = \frac{136.1 \times 10^{-3}}{136.1} \times \frac{1000}{100} = 0.01 \text{ mol/L (M)}$$

Hence molarity of PO_4^{3-} = 0.01 mol/L

1 mol of PO_4^{3-} equivalent to 95g PO_4^{3-}

0.01 mol $PO_4^{3-} \approx$ 0.95g PO_4^{3-}

So, there is 0.95g PO_4^{3-} in 1000ml solution

So, there is 9.5×10^{-4} g PO_4^{3-} in 1ml solution

Or, 950 µg PO_4^{3-} in 1ml solution

The standard stock solution contains ≈ 1000 µg PO_4^{3-} /g

The standard stock solution was then diluted in appropriate concentration and their absorbance measured (Table 2.17).

Calibration

The standard stock solution was then diluted to working solutions for calibration. One part of the 'Working Reagent' was added to 10 parts of the standard solution. Absorbance for each

concentration was measured at 885ηm after 20 minutes using a 10cm cuvette (Table 2.16) and a calibration curve is obtained (Figure 2.17).

Table 2.17 Concentration of PO_4^{3-} and their absorbance.

Concentration of PO_4^{3-} (μg/g)	Absorbance
0.00	0.00
0.01	0.023
0.05	0.042
0.10	0.049
0.50	0.123
1.00	0.231

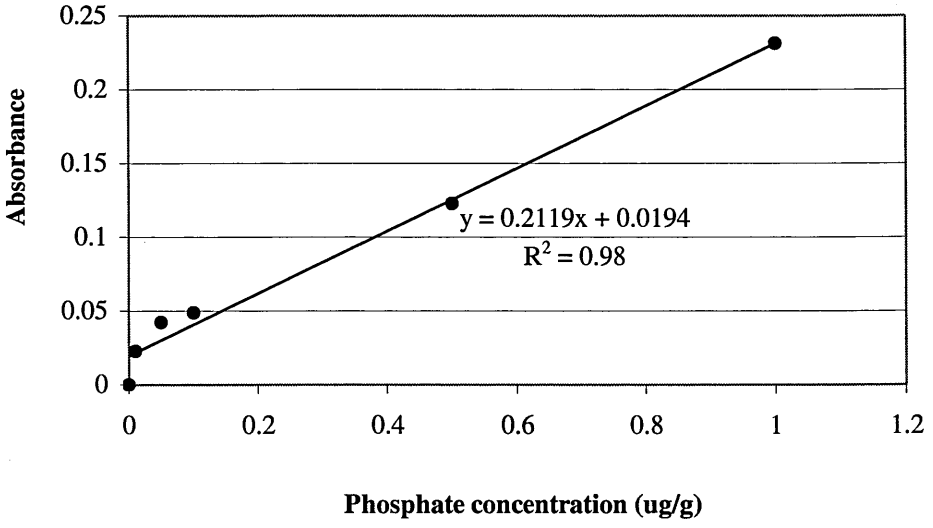


Figure 2.17 Absorbance versus concentration of PO_4^{3-}.

From the equation of the calibration line, y = 0.2119x + 0.0194, concentration of a sample can be calculated as:

$$\text{Phosphate concentration (μg/g)} = \frac{\text{Absorbance (y)} - 0.0194}{0.2119}$$

2.2.9 Data analysis

Deviation

Percentage uncertainty is used to measure the analytical reproducibility. Deviation is the difference between an observed value and the sample means (Wardlaw, 1985). Mean deviation (MD) of a set of observations x_1, x_2, ... x_N is the mean of the absolute deviations from the mean, and equals $1/N \ |\Sigma xi - x|$ (Clarke and Cooke, 1998). Probable uncertainty = $MD/\Sigma n$ of a measurement is reported as mean±probable uncertainty. Percentage uncertainty = probable uncertainty/average value x 100% (http://www.bearwoodphysics.com/uncertainties.htm and http://spiff.rit.edu/classes/phys376/uncert.html).

Correlation

Correlation is used to examine the relationship between two or more quantitative variable (Weiss, 2002). The coefficient of variation r takes only the interval –1 to +1. It takes the value ±1 when there is an exact straight-line relation Y=mX+c connecting the two variants. When m is positive, r=+1 and when m is negative, r=-1. When there is no correlation r will be 0. Where there is strong correlation r will be near ±1 (Clarke and Cooke, 1998).

ANOVA

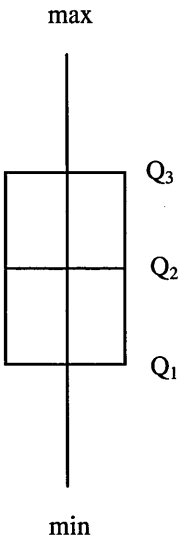
The analysis of variance (ANOVA) is used to compare the means of more than two populations. The assumptions for one-way ANOVA are: samples taken from the populations are independent of one another; for each population, the variable under consideration is normally distributed; and the standard deviations of the variable under consideration are the same for all the populations (Weiss, 2002).

Regression analysis

The straight line that best fits a set of data points is the one having the possible sum of squared errors. Regression line is the straight line that best fits a set of data points according to the least-squares criterion, and the regression equation is the equation of the regression line (Weiss, 2002).

Box plots

A box plot is based on the five-number summary of Min, Q_1, Q_2, Q_3 and Max in increasing order. It is used to show a graphical display of the centre and variation of a data set (Weiss, 2002).



where

Q_1, the first quartile, is the number that divides the bottom 25% of the data from the top 75%

Q_2, the second quartile, is the median

Q_3, the third quartile, is the number that divides the bottom 75% of the data from the top 25%

CHAPTER 3: RESULTS

Results for all the experiments carried out for the sediment samples from Loch Creran and Loch Etive are given in this chapter: environmental parameters (Section 3.1), lignin (Section 3.2), oxygen uptake rates (Section 3.3), percentage organic matter calculated by loss on ignition (Section 3.4), stable carbon isotopic ratios (Section 3.5), total carbon (TC), total nitrogen (TN) and total organic carbon (TOC) (Section 3.6), and phosphate contents (Section 3.7). Relationships between all these results with the lignin parameters are also determined (Section 3.8). Throughout this chapter, all the important findings are summarised at the end of each section, and these serve as the subjects for discussion in the following chapter.

3.1 ENVIRONMENTAL PARAMETERS

The air temperature, wind speed and rainfall, sedimentation rate and the River Creran water flow rate are discussed in this section.

3.1.1 Temperature, wind speed and rainfall

Environmental data consisting of air temperature, wind speed and rainfall were obtained from the Meteorology (MET) Office. These parameters were recorded at the Dunstaffnage station (altitude=3 metres, latitude=56:45N, longitude=05:44W), in Dunbeg, Oban, as this is the nearest station to our sampling locations, Loch Creran and Loch Etive. The monthly results were averaged and illustrated in Figure 3.1.

In Figure 3.1a, the mean of the maximum and minimum monthly air temperatures obtained from the Meteorology (MET) office are plotted against the "expected temperatures for that time of the year" obtained from: http://www.metoffice.com/climate/uk/averages/19712000/index.html. The terms used to describe the temperature obtained from the MET Office relative to that obtained for

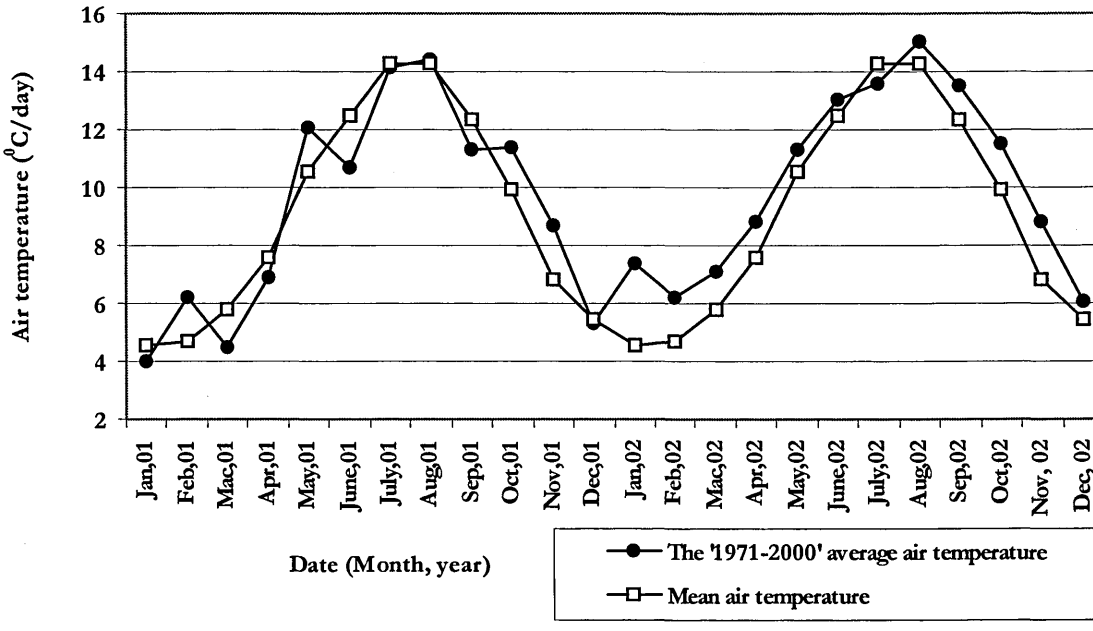
a certain time of the year was obtained from: http://www.metoffice.com/weather/europe/uk/guide.htm. The mean air temperatures recorded at the Dunstaffnage station are mostly "quite normal", as comparison with the expected temperatures show the same distribution pattern (Figure 3.1a). For both 2001 and 2002, the recorded temperatures (from the MET Office) peaked in August (14.33 and 15.01°C, respectively).

In 2001, the highest mean wind speed occurred in February (9 knots/day), October (9.14 knots/day) and November (9.05 knots/day; Figure 3.1b). In 2002, high wind speed occurred from January to March, peaking in February (11.11 knots/day). The wind speeds can also be depicted in Beaufort Number. The Beaufort chart is obtained from: http://www.eurometeo.com/english/read/doc_beaufort and http://www.r-p-r.com/beaufort.htm.

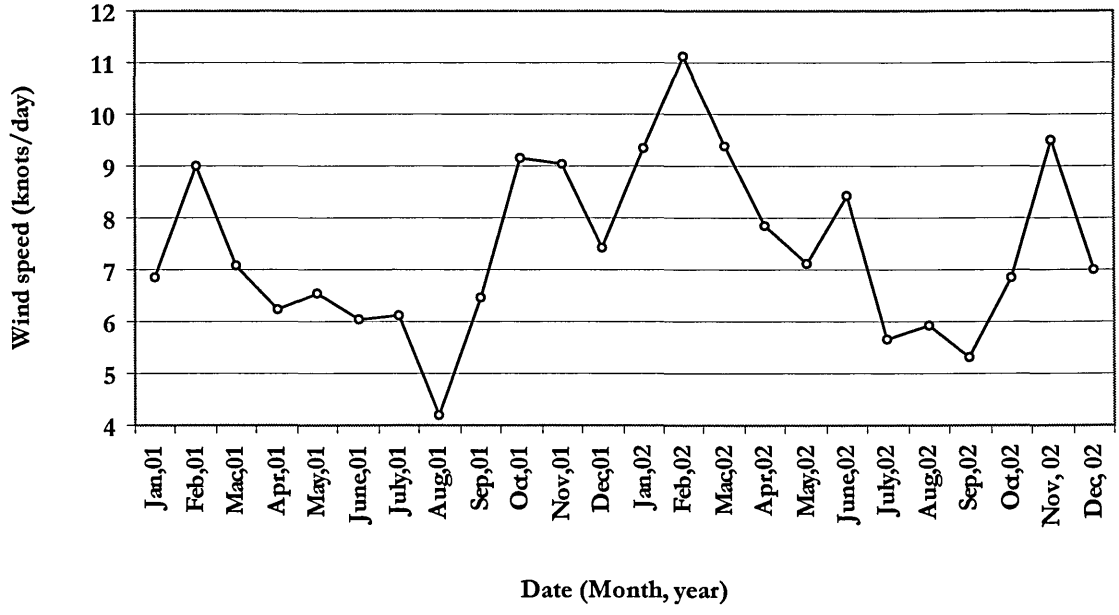
The highest rainfall in 2001 was recorded as 6.234 mm/day (0.26 mm/hour) in October. According to the "MET Office Customer Centre (customercentre@metoffice.com)", rainfall is described as slight, moderate and heavy. Slight is <0.1mm to 0.5mm per hour, moderate is 0.6 to 3.9mm per hour, heavy is 4mm plus per hour. Hence the rainfalls recorded throughout the years (Figure 3.1c) were mostly considered "slight".

Air temperature is the best parameter to study the effect of seasonal variations as high or low wind speed and rainfall could occur at anytime of the year. Regression analysis shows only significant correlation between wind speed and temperature ($r=-0.51$, $r^2=0.26$, $p<0.05$, $n=24$). There is no significant correlation between rainfall and air temperature ($r=-0.10$, $r^2=0.01$, $p>0.05$, $n=24$) and between wind speed and rainfall ($r=0.32$, $r^2=0.10$, $p>0.05$, $n=24$).

(a) Air temperature. The average temperatures for these two years (obtained from the MET Office) are also compared to the average values obtained from the "1971-2000 averages".



(b) Wind speed.



(c) Rainfall.

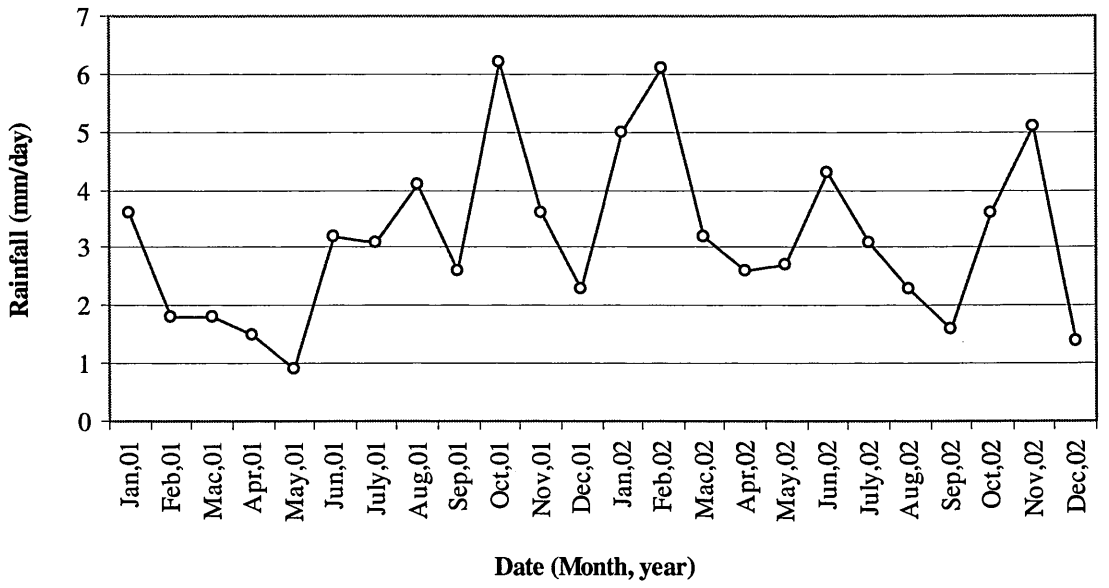


Figure 3.1 Environmental parameters.

The bottom and surface water temperatures, and the air temperatures were also measured during some sampling trips (Table 3.1). There are some differences between these measurements with the mean values obtained from the MET Office (in parentheses in Table 3.1). Air temperatures ranged from the winter low of 2°C in December 2001 to the summer high of 19.5°C in September 2002. Surface water temperatures in Loch Creran ranged from 5.6°C in March 2002 to 14.6°C in September 2002. Bottom water temperatures ranged from 7.0°C in March 2002 to 14.6°C in September 2002.

Our measurements of the bottom and surface water, and air temperatures at LC1 are illustrated in Figure 3.2. The surface air temperature reached the lowest value in December 2001, and in the summer from June to September 2002 was above the water temperatures. The bottom water temperature showed slower decrease in the winter. Form Figure 3.2 it is observed that in late summer, the surface and bottom water temperatures finally achieved equilibrium in September 2002.

Table 3.1 Surface and bottom water and air temperatures for Loch Creran.

Sampling date	Locations	Bottom water temperatures (°C)	Surface water temperatures (°C)	Air temperatures (°C)
12.12.2001	LC1	11.2	8.0	2.0 (5.31)
7.3.2002	LC1	7.0	5.6	7.0 (7.11)
21.3.2002	LC3	8.0	7.5	11.2
7.5.2001	LC1	9.5	-	-
	LC3	9.6	12.8	15.4 (11.33)
4.6.2002	LC1	10.6	11.4	14.5 (13.04)
2.9.2002	LC1	15.3	14.5	17.5
	LC2	14.6	14.6	16.5
30.9.2002	LC1	14.5	14.5	19.5 (13.50)
	LC3	14.5	-	-
	LC6	14.5	-	-

The air temperatures in parentheses are mean values obtained from the MET office. There seems to be some differences between these values and our measurements, as the values from the MET Office are means of the daily values of that month.

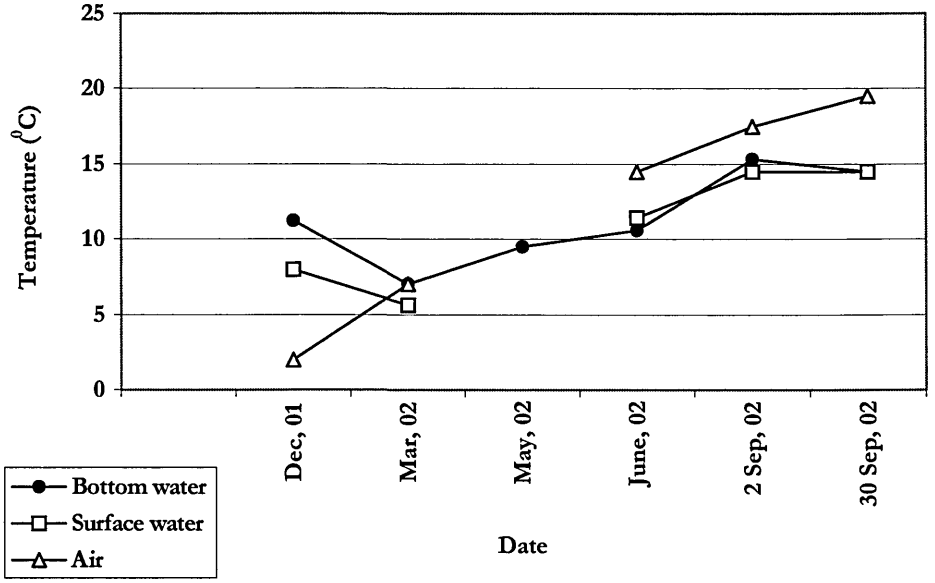


Figure 3.2 Surface and bottom water, and air temperatures at LC1. These are the measurements obtained for LC1 from Table 3.1.

3.1.2 Sedimentation rate

The sedimentation rate was calculated from the sediment trap samples as the sediment dry weight/areas of the collecting tubes/total time (day) of the trap deployment (also explained in section 2.2.1.2). Results are illustrated in Figure 3.3. The sedimentation rates at LC1 ranged from 3.67 to 31.48 g/m^2/day. Sedimentation rates were high in April (21.38 g/m^2/day) and June (15.91 g/m^2/day) and highest in August (31.48 g/m^2/day), most probably because there was high rainfall in the months preceding these few months. High rainfall occurring in February and June 2002 transported terrestrial debris into Loch Creran, resulting in high deposition and sedimentation rate in the months following. The sedimentation rate will be used in the determination of carbon budgets. The mean sedimentation rate = (6.97+4.10+8.13+9.73+21.38+6.55+15.91+3.67+31.48+10.43+3.9)/11 = 11.11g/m^2/d.

3.1.3 River Creran water flow rate

The River Creran water flow rate was calculated by dividing the distance travelled by a free floating stick of the width of the bridge of 3.5m wide with the time for the stick to travel the width. At every 1m intervals along the bridge, a stick was introduced onto the water and the time for the stick to travel the width of the bridge was recorded (Section 2.2.1.3). Hence, the distance at the x-axis of Figure 3.4 represents the distance from one end of the bridge, at every 1m-interval, along the bridge, to the other far end of the bridge. From Figure 3.4, the river flow rate was higher in March 2003 than October 2002, as the heavy rainfall and snowmelt at the end of the winter months would have induced more rapid river flow rate in March 2003. October 2001 seems to have the same flow rate as October 2002. The River Creran water flow rates serve only for information as there is not enough data to warrant further discussion.

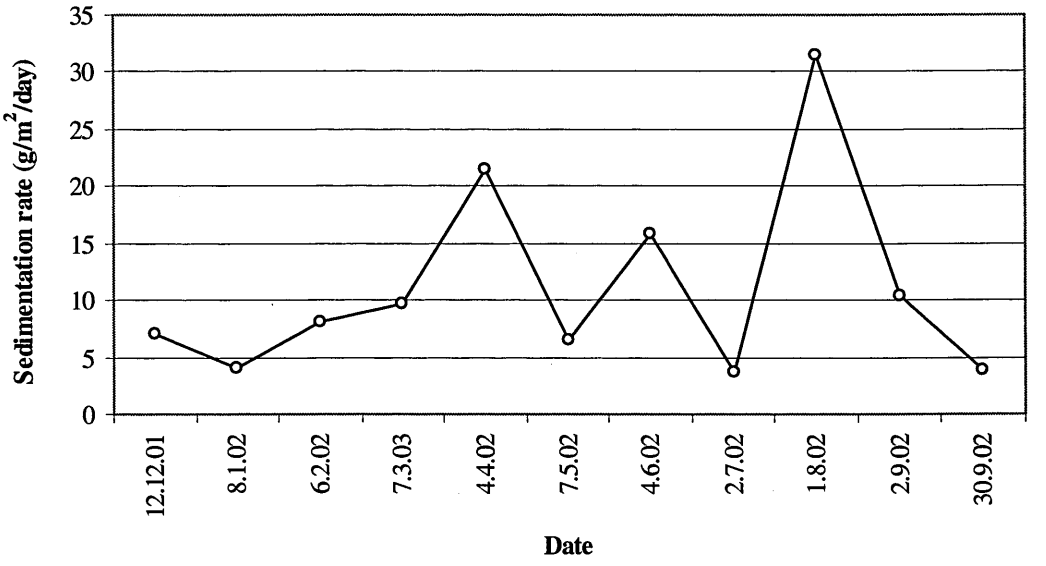


Figure 3.3 Sedimentation rate.

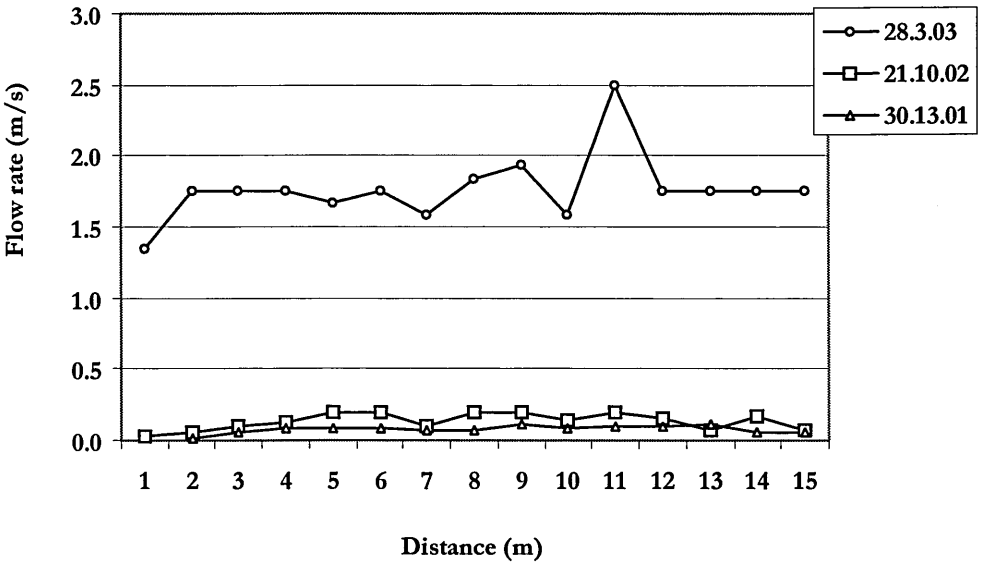


Figure 3.4 River Creran water flow rate. The "distance" here means the distances from one of the far end of the bridge, measured at every 1m intervals, along the bridge (also refer Figure 2.7), towards the other far end of the bridge.

3.2 LIGNIN

Of the 12 lignin phenols, only eight (vanillin, acetovanillone, syringaldehyde, acetosyringone, syringic acid, vanillic acid, p-coumaric acid and ferulic acid) were used to calculate the total lignin concentration (mg/g dry weight of sediment sample). The sum of these eight lignin phenols will be known as total lignin (mg/g) or lignin (mg/g) or lignin abundance (Sections 1.5.1.5, 2.2.3.9 and 2.2.3.11). An example of a chromatogram of a sediment sample is given in **Appendix 2.** Ethyl vanillin, the internal standard was not used in the calculation of the total lignin. The three p-hydroxyl phenols (also known as "P") consisting of p-hydroxybenzaldehyde, p-hydroxyacetophenone and p-hydroxybenzoic acid, are also not included in the calculation of total lignin as these could be the oxidation products of non-vascular plants. For example, Hedges and Parker (1976) analysed the blue-green alga *Anacystis nidulans* and the brown alga *Sargassum sp.*and found that these three p-hydroxyphenols were present. However, these three phenols might be used to indicate the input of marine organic matter instead.

Vanillyl phenols (V) consist of vanillin (an aldehyde), acetovanillone (a ketone), and vanillic acid (a carboxylic acid). Syringyl phenols (S) consist of syringaldehyde (aldehyde), acetosyringone (ketone) and syringic acid (carboxylic acid). Cinnamyl phenols (C) consist of the p-coumaric and ferulic acids. The S/V and C/V ratios are used to distinguish the plant sources in the sediment samples (Section 1.5.1.5). Gymnosperm tissues produce only vanillyl phenols. Angiosperm produce both syringyl and vanillyl phenols, hence the S/V ratios are greater than zero. The C/V ratio is used to distinguish between non-woody and woody tissues, as only the non-woody tissues of angiosperm and gymnosperm produce cinnamyl phenols (Hedges and Mann, 1979a). The vanillic acid to vanillin (Ad/Al)v and the syringic acid to syringaldehyde (Ad/Al)s ratios are used to determine the extent to which terrestrial organic matter have undergone degradation. Both (Ad/Al)v and (Ad/Al)s are discussed, but later only (Ad/Al)v will be used.

3.2.1 Lignin in Loch Creran

This section includes detail studies of each lignin parameter in individual sampling location in Loch Creran. Lignin results for all the sampling locations in the lochs are given in Table 3.2. Results are mean values of a duplicate analysis of sediments from the same sampling trip, one analysis from one sediment core. Duplicate analysis is carried out as the reproducibility is within the ranges when several analyses were carried our for the same sediment sample. Data analyses were performed only for total lignin, syringyl, vanillyl and cinnamly phenol groups, the S/V, C/V and (Ad/Al)v. Data analysis was not performed on individual lignin phenols, as these make up the components of the syringyl, vanillyl and cinnamyl phenol groups.

3.2.1.1 Lignin at individual locations

Generally the vanillyl phenols are present in the greatest abundance, followed by the syringyl, cinnamyl and p-hydroxyl phenols present in either higher or lower abundances than one another. For the p-hydroxyl phenols, their components decrease in this order: p-hydroxybenzoic acid>p-hydroxybenzaldehyde>p-hydroxyacetophenone. For the vanillyl and syringyl groups, the sediments yielded more aldehydes than the acid and ketone groups. Similar circumstances were also observed by Hedges and Parker (1976), where the syringyl and vanillyl phenols were produced primarily as aldehydes for most of their sediment samples. For the cinnamyl phenols, ferulic acid usually occurs in more abundance than p-coumaric acid. The ratios between the acid and aldehyde groups are given as (Ad/Al)v and (Ad/Al)s. There are strong correlations between both ratios for the sediment trap, LC0 and LC3 sediments (Table 3.3). Only (Ad/Al)v is used here as the vanillyl phenols are present in the greatest abundance. The vanillyl group has strong positive correlations with total lignin for sediments from all sampling locations hence there are more strong correlation between (Ad/Al)v and total lignin compared to between (Ad/Al)s and total lignin, as shown in Table 3.3.

Table 3.2 Lignin parameters for all locations in Loch Creran.

(a) Sediment trap.

Lignin parameters	Sampling date with the lignin concentration (mg/g dry sediment weight)												
	12.12.01	8.1.02	6.2.02	7.3.02	4.4.02	7.5.02	4.6.02	2.7.02	1.8.02	2.9.02	30.9.02	14.11.02	12.12.02
(P): p-Hb	0.0228	0.0207	0.0255	0.0259	0.0536	0.0076	0.0110	0.0415	0.0193	0.0231	0.0428	0.0219	0.0146
p-Ha	0.0051	0.0105	0.0134	0.0138	0.0487	0.0072	0.0325	0.0059	0.0059	0.0081	0.0074	0.0090	0.0069
p-Hba	0.0559	0.0492	0.0705	0.0652	0.1246	0.1537	0.0301	0.1876	0.0394	0.0471	0.0774	0.0502	0.0364
(V): Van	0.0634	0.0544	0.0681	0.0702	0.0181	0.0124	0.0170	0.0197	0.0195	0.0372	0.0271	0.0408	0.0304
Acetovan	0.0330	0.0411	0.0470	0.0429	0.0343	0.0342	0.0370	0.0353	0.0259	0.0314	0.0393	0.0372	0.0249
Va	0.0479	0.0513	0.0690	0.0726	0.0859	0.0139	0.0256	0.1500	0.0284	0.0445	0.0809	0.0591	0.0389
(S): Syring	0.0483	0.0480	0.0565	0.0604	0.0172	0.0117	0.0530	0.0136	0.0251	0.0430	0.0332	0.0484	0.0325
Acetosy	0.0210	0.0195	0.0256	0.0264	0.0172	0.0501	0.0155	0.0179	0.0107	0.0155	0.0179	0.0181	0.0148
Sa	0.0246	0.0292	0.0355	0.0364	0.0445	0.0356	0.0168	0.0691	0.0185	0.0252	0.0432	0.0322	0.0212
(C): p-Cou	0.0406	0.0422	0.0786	0.0651	0.0237	0.0126	0.0305	0.0325	0.0159	0.0289	0.0442	0.0358	0.0294
Fe	0.0584	0.0495	0.0957	0.0843	0.0242	0.0147	0.0212	0.0279	0.0193	0.0370	0.0578	0.0466	0.0348
P	0.0838	0.0804	0.1094	0.1049	0.2269	0.1685	0.0736	0.2350	0.0646	0.0783	0.1276	0.0811	0.0579
V	0.1433	0.1468	0.1841	0.1857	0.1383	0.0605	0.0796 <	0.2050 >	0.0738 <	0.1131 <	0.1473	0.1371	0.0942
S	0.0939	0.0967	0.1176	0.1232 >	0.0789	0.0974	0.0853	0.1006 >	0.0543	0.0837	0.0943	0.0987	0.0685
C	0.0990	0.0917	0.1743	0.1494 >	0.0479 >	0.0273 <	0.0517	0.0604 >	0.0352 <	0.0659 <	0.1020	0.0824	0.0642
Lignin	0.3362	0.3352	0.4760	0.4583 >	0.2651	0.1852	0.2166	0.3660 >	0.1633 <	0.2627 <	0.3436	0.3182	0.2269
S/V	0.6553	0.6587	0.6388	0.6634	0.5705	1.6100	1.0716	0.4907 <	0.7358	0.7401 >	0.6402 <	0.7199	0.7272
C/V	0.6909	0.6247	0.9468	0.8045 >	0.3465	0.4512 <	0.6495 >	0.2946	0.4740	0.5827	0.6925	0.6010	0.3631
(Ad/Al)v	0.7555	0.9430	1.0132	1.0342 <	4.7459	1.1210	1.5059 <	7.6142 >	1.4564	1.1962	2.9852	1.4485	1.2796
(Ad/Al)s	0.5093	0.6083	0.6283	0.6026	2.4872	3.0427	0.3170	5.0809	0.7371	0.5860	1.3012	0.6653	0.6532

P=p-hydroxyl phenols, V=vanillyl phenols, S=syringyl phenols; p-hb=p-hydroxybenzaldehyde, p-ha=p-hydroxyacetophenone, p-hba=p-hydroxybenzoic acid, van=vanillin, acetocan=acetovanillone, va=vanillic acid, syring=syingldehyde, acetosy=acetosyringone, sa=syringic acid, p-cou=p-coumaric acid, fe=ferulic acid; (Ad/Al)v=ratio of vanillic acid to vanillin and (Ad/Al)s=ratio of syringic acid to syringaldehyde.

(b) LC1.

Lignin parameters	Sampling date and sediment depth (in parentheses), with lignin concentration (mg/g)															
	Creran head		LC1(8.1.02)	6.2.02	7.3.02	7.5.02	4.6.02		2.7.02	1.8.02	2.9.02		30.9.02	14.11.02	12.12.02	
	0-1cm	9-10cm	0-1cm	0-1cm	0-1cm	0-1cm	0-1cm	9-10cm	0-1cm	0-1cm	0-1cm	9-10cm	0-1cm	0-1cm	0-1cm	9-10cm
(P) p-Hb	0.0032	0.0017	0.0207	0.0285	0.0135	0.0054	0.0159	0.0162	0.0118	0.0233	0.0268	0.0130	0.0076	0.0208	0.0208	0.0195
p-Ha	0.0030	0.0014	0.0117	0.0095	0.0101	0.0030	0.0091	0.0109	0.0074	0.0068	0.0125	0.0071	0.0096	0.0049	0.0063	0.0095
p-Hba	0.0066	0.0036	0.0500	0.0387	0.0355	0.0085	0.0415	0.0417	0.0353	0.0501	0.0530	0.0319	0.0152	0.0598	0.0451	0.0431
(V): Van	0.0049	0.0047	0.0370	0.0222	0.0279	0.0076	0.0382	0.0320	0.0251	0.0453	0.0517	0.0288	0.0268	0.0547	0.0496	0.0537
Acetovan	0.0191	0.0155	0.0338	0.0249	0.0215	0.0028	0.0273	0.0184	0.0241	0.0350	0.0259	0.0214	0.0252	0.0379	0.0303	0.0402
Va	0.0029	0.0023	0.0145	0.0172	0.0261	0.0041	0.0442	0.0304	0.0193	0.0145	0.0372	0.0256	0.0361	0.0642	0.0406	0.0462
(S) Syring	0.0038	0.0011	0.0255	0.0359	0.0226	0.0045	0.0364	0.0254	0.0218	0.0104	0.0320	0.0217	0.0272	0.0393	0.0365	0.0417
Acetosy	0.0023	0.0021	0.0113	0.0157	0.0131	0.0020	0.0162	0.0120	0.0131	0.0071	0.0130	0.0109	0.0190	0.0197	0.0175	0.0197
Sa	0.0025	0.0016	0.0218	0.0126	0.0141	0.0029	0.0206	0.0155	0.0102	0.0202	0.0198	0.0139	0.0243	0.0311	0.0214	0.0222
(C) p-Cou	0.0026	0.0016	0.0388	0.0268	0.0358	0.0031	0.0384	0.0389	0.0355	0.0386	0.0436	0.0342	0.0414	0.0375	0.0346	0.0398
Fe	0.0303	0.0188	0.0346	0.0281	0.0388	0.0014	0.0508	0.0421	0.0337	0.0255	0.0472	0.0387	0.0297	0.0410	0.0414	0.0529
P	0.0128	0.0067	0.0824	0.0767	0.0591	0.0169	0.0665	0.0688	0.0545	0.0802	0.0923	0.0520	0.0324	0.0855	0.0722	0.0721
V	0.0269	0.0185	0.0853	0.0643	0.0755>	0.0145<	0.1097	0.0808	0.0685	0.0948	0.1148	0.0758	0.0881	0.1568	0.1205	0.1401
S	0.0086	0.0048	0.0586	0.0642	0.0498>	0.0094<	0.0732	0.0529	0.0451	0.0377	0.0648	0.0465	0.0705	0.0901	0.0754	0.0836
C	0.0329	0.0204	0.0734	0.0549	0.0746>	0.0045<	0.0892	0.0810	0.0692	0.0641	0.0908	0.0729	0.0711	0.0785	0.0760	0.0926
Lignin	0.0684	0.0437	0.2173	0.1834<	0.1999	0.0284<	0.2721	0.2147	0.1828	0.1966	0.2704	0.1952	0.2297	0.3254	0.2719	0.3163
S/V	0.3197	0.2595	0.6870	0.9984	0.6596	0.6483	0.6673	0.6547	0.6584	0.3977	0.5645	0.6135	0.8002	0.5746	0.6257	0.5967
C/V	1.2230	1.1027	0.8605	0.8538	0.9881>	0.3103<	0.8131	1.0025	1.0102	0.6762	0.7909	0.9617	0.8070	0.5006	0.6307	0.6610
(Ad/Al)v	0.5918	0.4894	0.3919<	0.7748	0.9355	0.5395<	1.1571	0.9500	0.7689	0.3201	0.7195<	0.8889	1.3470	1.1737	0.8185<	0.8603
(Ad/Al)s	0.6579	1.4545	0.8549	0.3510	0.6239	0.6444	0.5659	0.6102	0.4679	2.9038	0.6188	0.6406	0.8934	0.7913	0.5863	0.5324

(c) LC0.

Lignin parameters	Sampling date and sediment depth (in parentheses), with lignin concentration (mg/g)				
	8.1.02 (0-1cm)	4.4.02 (0-1cm)	4.6.02 (0-1cm)	14.11.02 (0-1cm)	14.11.02 (9-10cm)
(P): p-Hb	0.0278	0.0261	0.0181	0.0188	0.0150
p-Ha	0.0148	0.0146	0.0087	0.0065	0.0059
p-Hba	0.0508	0.0748	0.0448	0.0482	0.0520
(V): Van	0.0540	0.0566	0.0506	0.0571	0.0459
Acetovan	0.0278	0.0377	0.0372	0.0362	0.0440
Va	0.0464	0.0753	0.0469	0.0494	0.0622
(S): Syring	0.0388	0.0467	0.0464	0.0427	0.0397
Acetosy	0.0147	0.0254	0.0218	0.0173	0.0193
Sa	0.0247	0.0424	0.0248	0.0240	0.0297
(C): p-Cou	0.0391	0.0502	0.0481	0.0388	0.0380
Fe	0.0522	0.0328	0.0658	0.0540	0.0475
P	0.0934	0.1155	0.0716	0.0735	0.0729
V	0.1282	0.1696	0.1347	0.1427	0.1521
S	0.0782	0.1145	0.0930	0.0840	0.0887
C	0.0913	0.0830	0.1139	0.0928	0.0855
Lignin	0.2977	0.3671	0.3416	0.3195	0.3263
S/V	0.6100	0.6751	0.6904 >	0.5886	0.5832
C/V	0.7123 >	0.4894 <	0.8456 >	0.6503	0.5621
(Ad/Al)v	0.8593	1.3304	0.9269	0.8651 <	1.3551
(Ad/Al)s	0.6366	0.9080	0.5345	0.5621	0.7481

(d) LC2.

Lignin parameters	Sampling date and sediment depth (in parentheses), lignin concentration (mg/g)		
	4.4.02 (0-1cm)	2.9.02 (0-1cm)	2.9.02 (9-10cm)
(P): p-Hb	0.0140	0.0122	0.0155
p-Ha	0.0081	0.0037	0.0069
p-Hba	0.0390	0.0223	0.0293
(V): Van	0.0357	0.0216	0.0246
Acetovan	0.0370	0.0197	0.0224
Va	0.0466	0.0153	0.0216
(S): Syring	0.0281	0.0094	0.0185
Acetosy	0.0183	0.0054	0.0091
Sa	0.0251	0.0100	0.0122
(C): p-Cou	0.0269	0.0177	0.0307
Fe	0.0212	0.0213	0.0296
P	0.0611	0.0382	0.0517
V	0.1193 >	0.0566	0.0686
S	0.0715 >	0.0248	0.0298
C	0.0481	0.0390	0.0603
Lignin	0.2389 >	0.1204	0.1687
S/V	0.5993 >	0.4382 <	0.5802
C/V	0.4032 <	0.6890	0.8790
(Ad/Al)v	1.3053	0.7083 <	0.8780
(Ad/Al)s	0.8932	1.0638	0.6595

(e) LC3.

Lignin parameters	Sampling date and sediment depth (in parentheses), with lignin concentration (mg/g)		
	7.5.02 (0-1cm)	30.9.02 (0-1cm)	30.9.02 (9-10cm)
(P): p-Hb	0.0039	0.0049	0.0167
p-Ha	0.0024	0.0044	0.0039
p-Hba	0.0091	0.0213	0.0147
(V): Van	0.0076	0.0066	0.0037
Acetovan	0.0153	0.0211	0.0166
Va	0.0042	0.0104	0.0053
(S): Syring	0.0055	0.0072	0.0065
Acetosy	0.0023	0.0045	0.0030
Sa	0.0027	0.0108	0.0038
(C): p-Cou	0.0079	0.0035	0.0090
Fe	0.0025	0.0158	0.0087
P	0.0154	0.0306	0.0353
V	0.0271 <	0.0381 >	0.0256
S	0.0105	0.0225	0.0133
C	0.0104	0.0193 <	0.0377
Lignin	0.0480 <	0.0799	0.0766
S/V	0.3875	0.5906	0.5196
C/V	0.3838	0.5066 <	1.4727
(Ad/Al)v	0.5526	1.5758 >	1.4324
(Ad/Al)s	0.4909	1.5000	0.5846

(f) LC5.

Lignin parameters	Sampling date and sediment depth (in parentheses), with lignin concentration (mg/g)			
	6.2.02 (0-1cm)	2.7.02 (0-1cm)	12.12.02 (0-1cm)	12.12.02 (9-10cm)
(P): p-Hb	0.0206	0.0154	0.0276	0.0277
p-Ha	0.0036	0.0125	0.0042	0.0032
p-Hba	0.0058	0.0349	0.0132	0.0053
(V): Van	0.0072	0.0310	0.0180	0.0065
Acetovan	0.0214	0.0257	0.0206	0.0114
Va	0	0.0307	0.0101	0.0033
(S): Syring	0.0031	0.0292	0.0119	0.0037
Acetosy	0	0.0119	0.0058	0.0020
Sa	0.0013	0.0146	0.0061	0.0025
(C): p-Cou	0	0.0327	0.0098	0.0024
Fe	0	0.0362	0.0255	0.0218
P	0.0300	0.0628	0.0450	0.0362
V	0.0286 <	0.0874	0.0481	0.0212
S	0.0044 <	0.0557	0.0238	0.0082
C	0 <	0.0689	0.0353	0.0242
Lignin	0.0330 <	0.2120 >	0.1072 >	0.0536
S/V	0.1538 <	0.6373	0.4948	0.3868
C/V	0 <	0.7883	0.7339	1.1415
(Ad/Al)v	0 <	0.9903 >	0.5611 >	0.5077
(Ad/Al)s	0.4194	0.5000	0.5042	0.6757

(g) LC6.

Lignin parameters	Sampling date and sediment depth (in parentheses), with lignin concentration (mg/g)			
	21.3.02 (0-1cm)	1.8.02 (0-1cm)	30.9.02 (0-1cm)	30.9.02 (9-10cm)
(P): p-Hb	0.0033	0.0030	0.0171	0.0045
p-Ha	0.0036	0.0024	0.0033	0.0031
p-Hba	0.0078	0.0083	0.0076	0.0069
(V): Van	0.0062	0.0054	0.0051	0.0039
Acetovan	0.0057	0.0114	0.0172	0.0079
Va	0.0069	0.0035	0.0042	0.0037
(S): Syring	0.0055	0.0026	0.0024	0.0020
Acetosy	0.0028	0.0013	0.0028	0.0015
Sa	0.0028	0.0023	0.0021	0.0029
(C): p-Cou	0.0042	0.0042	0.0120	0.0059
Fe	0.0027	0.0218	0.0233	0.0016
P	0.0147	0.0137	0.0280	0.0145
V	0.0188 <	0.0203	0.0265 >	0.0155
S	0.0111 >	0.0062	0.0073	0.0064
C	0.0069 <	0.0260 <	0.0353 >	0.0075
Lignin	0.0368	0.0525 <	0.0691 >	0.0294
S/V	0.5904 >	0.3054	0.2755	0.4129
C/V	0.3670 <	1.2808	1.3321	0.4839
(Ad/Al)v	1.1129	0.6481	0.8235 <	0.9487
(Ad/Al)s	0.5091	0.8846	0.8750	1.4500

Results are mean values of duplicate analyses of the sediment from the same sampling trip, one analysis from one sediment core. The symbols ">" and "<" indicate that the lignin parameter is significantly higher or lower than that parameter in the subsequent month.

As V, S and C were used to calculate the total lignin concentration, strong positive correlations between V, S, C and total lignin were observed (see correlation table, Table 3.3). Correlation between the C groups with other lignin groups was weaker for sediments from LC0, LC2 and LC3. This could be due to the hydrodynamic sorting process distinguishing the woody from the non-woody tissues, as will be discussed in the next chapter. The S group had the poorest correlation with other lignin groups for sediments from LC6. There is strong correlation of the vanillyl phenols with total lignin from all the sampling locations. The P group has the strongest correlation with other lignin groups only for sediments from LC2 and LC5, most probably because the p-hydroxyl phenols could be due to the oxidation products of both terrestrial and marine materials. The vanillyl phenols have the strongest correlation with total lignin for sediments from the sediment trap, LC1, LC0, LC2, LC5 and LC6. The greater stability of the

vanillyl compared to the syringyl and cinnamyl phenols (Hedges *et al.*, 1985; Ertel *et al.*, 1986; Hedges *et al.*, 1986; Hedges *et al.*, 1988c; Lobbes *et al.*, 2000; Dittmar and Lara, 2001) could be the reason why the vanillyl phenols, and hence the (Ad/Al)v ratio exhibited more constant and stronger correlation with total lignin.

Table 3.3 Correlations between lignin parameters for the 0-1 and 9-10cm sediment layers for all the sampling duration.

Locations	Coefficient of variation (r values, in numbers) between the V, S, C and (Ad/Al)v, (Ad/Al)s with lignin							
Sediment trap		V	S	C	Lignin		(Ad/Al)v	Lignin
	V				0.92	(Ad/Al)v		0.08
	S	0.69			0.85	(Ad/Al)s	0.85	-0.04
	C	0.71	0.75		0.92			
	P	0.37	0.21	-0.19	0.13			
LC1		V	S	C	Lignin		(Ad/Al)v	Lignin
	V				0.97	(Ad/Al)v		0.45
	S	0.88			0.94	(Ad/Al)s	-0.46	-0.06
	C	0.79	0.76		0.90			
	P	0.68	0.57	0.66	0.68			
LC0		V	S	C	Lignin		(Ad/Al)v	Lignin
	V				0.80	(Ad/Al)v		0.61
	S	0.86			0.65	(Ad/Al)s	0.86	0.58
	C	-0.62	-0.23		-0.04			
	P	0.56	0.65	-0.54	0.44			
LC2		V	S	C	Lignin		(Ad/Al)v	Lignin
	V				0.97	(Ad/Al)v		0.99
	S	0.99			0.95	(Ad/Al)s	-0.19	-0.32
	C	0.10	0.01		0.33			
	P	0.90	0.86	0.52	0.98			
LC3		V	S	C	Lignin		(Ad/Al)v	Lignin
	V				0.49	(Ad/Al)v		0.99
	S	0.94			0.75	(Ad/Al)s	0.67	0.65
	C	-0.30	0.03		0.69			
	P	0.18	0.50	0.88	0.95			
LC5		V	S	C	Lignin		(Ad/Al)v	Lignin
	V				0.98	(Ad/Al)v		0.91
	S	0.99			0.99	(Ad/Al)s	0.31	-0.11
	C	0.90	0.96		0.97			
	P	0.96	0.99	0.98	0.99			
LC6		V	S	C	Lignin		(Ad/Al)v	Lignin
	V				0.97	(Ad/Al)v		-0.59
	S	-0.04			-0.24	(Ad/Al)s	-0.21	-0.31
	C	0.90	-0.45		0.97			
	P	0.88	-0.09	0.74	0.81			

There is no discernible trend in all the lignin parameters at individual locations throughout the year. Regression studies between total lignin at LC1 with the environmental parameters show no significant correlation: lignin versus rainfall ($r=0.34$, $r^2=0.10$, $p>0.05$, $n=10$); lignin versus air

temperature ($r=0.03$, $r^2=0.00$, $p>0.05$, $n=10$); lignin versus wind speed ($r=0.37$, $r^2=0.14$, $p>0.05$, $n=10$). For the sediment trap samples there is significant correlation between lignin and wind speed ($r^2=0.34$, $p<0.05$, $n=13$), but no significant correlations between lignin and rainfall ($r^2=0.00$, $p>0.05$, $n=13$) and between lignin and temperature ($r^2=0.27$, $p>0.05$, $n=13$). Lignin input is affected by seasonal changes, but apparently not by meteorological changes, as some of the lignin materials might be retained within the rivers, and some have travelled a distance. So, high lignin concentration does not correlate strongly with high rainfall.

One interesting feature is that for some of the sediments the total lignin and (Ad/Al)v were higher in the 0-1cm compared to the 9-10cm layers, while the opposite occurred for some sediment. The total lignin and the (Ad/Al)v are higher in the 0-1cm than the 9-10cm layers for the Creran head, LC1 (4.6.02), LC3 (30.9.02) and LC5 (12.12.02) sediments. For the LC1 (2.9.2002) and LC6 (30.9.2002) sediments the total lignin is higher but (Ad/Al)v is lower in the 0-1cm compared to the 9-10cm sediment layer. All these indicate that at times, the particles sedimentation rate was so high that the organic matter is deposited in their fresher stage while not yet undergone degradation. At times, the sedimentation rates were slower enabling the organic matter in the water column to degrade while settling down, hence resulting in more degraded materials settling on the fresher sediments.

3.2.1.2 Sediment depth profiles

Lignin depth profile was investigated by subjecting each layer from the core from LC1 collected on the 4.6.2002 to the CuO oxidation process and results are shown in Table 3.4. Data analysis using single factor ANOVA shows mostly no significant difference ($p>0.05$) for all the lignin parameters between each subsequent months. Significant difference in a lignin parameter between two subsequent sampling months is shown by either the ">" or "<" signs. Overall there is no significant trend for the lignin parameters down the core. Hedges and Parker (1979b) had also found relatively uniform lignin parameters with depth hence the mean values for these

parameters were compared with regard to distance from offshore. In future analysis only the 0-1cm and 9-10cm layers were used.

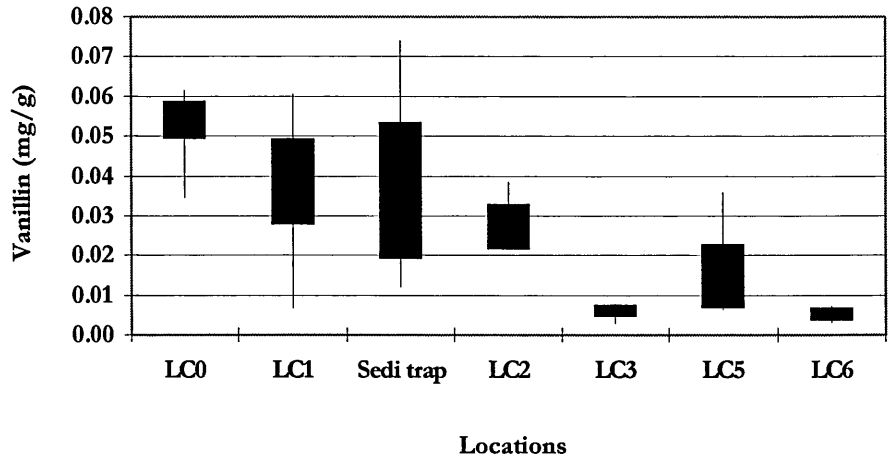
Table 3.4 Sediment lignin depth profile for LC1 (4.6.2002) sample. Significantly higher or lower (ANOVA: $p<0.05$) than the parameter in the following depth are shown by these signs: ">" and "<".

Depth (cm)	Total lignin (mg/g)	S	V	C	S/V	C/V	(Ad/Al)v
0-1	0.2720	0.0732	0.1096	0.0892	0.6674>	0.8137	1.1567>
1-2	0.1692	0.0372	0.0885	0.0435	0.4317	0.5260	0.2260
2-3	0.1334	0.0308	0.0662	0.0365	0.4664<	0.5520	0.1936<
3-4	0.2015	0.0799	0.0786	0.0429	1.0163	0.4551	0.5536
4-5	0.1863	0.0742>	0.0736	0.0386	1.0144>	0.5204	0.5542
5-6	0.1223	0.0268	0.0583<	0.0372	0.4589	0.6364	0.5667
6-7	0.1444	0.0278	0.0784>	0.0383	0.3541	0.4872	0.5879
7-8	0.1346	0.0512	0.0505	0.0329	1.0151	0.6530	0.6370
8-9	0.1508	0.0568	0.0626	0.0314<	0.9104>	0.5007<	0.5953
9-10	0.2146	0.0529	0.0808	0.0809	0.6552	1.0021	0.9492

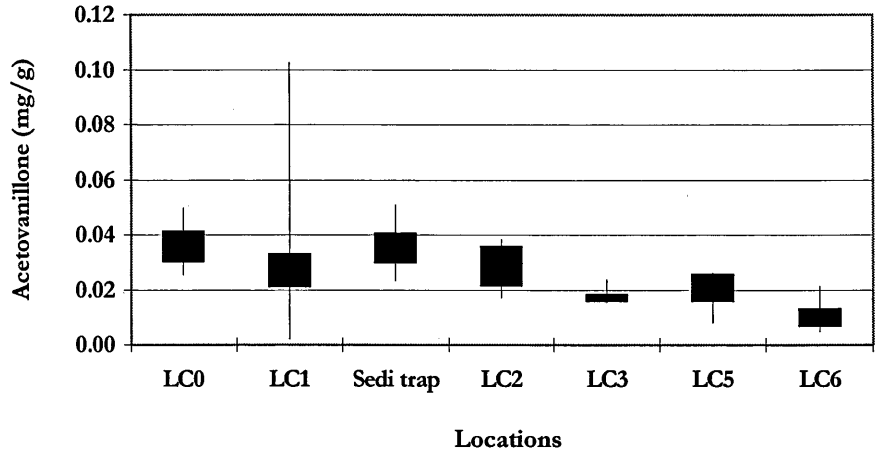
3.2.1.3 Lignin along the transect of Loch Creran

All individual lignin concentrations also decrease from the head to the mouth of the loch, as seen from the box plots for each lignin in Figure 3.5. Hedges and Parker (1976) found that the major oxidation products of the non-vascular plants studied were benzoic acid, hydroxybenzoic acids and p-hydroxybenzaldehyde. The box plots for the three p-hydroxyl phenols p-hydroxybenzaldhyde, p-hydroxyacetophenone and p-hydroxybenzoic acid plotted in Figure 3.5 (i-k) all demonstrate slight decrease from the head to the mouth of the loch. Hence the implication here is that these phenols are not derived from marine plants, because if they do the increase would be from the head towards the mouth of the loch. However, since they could be the oxidation products of non-vascular plants, they are not used to calculate the total lignin.

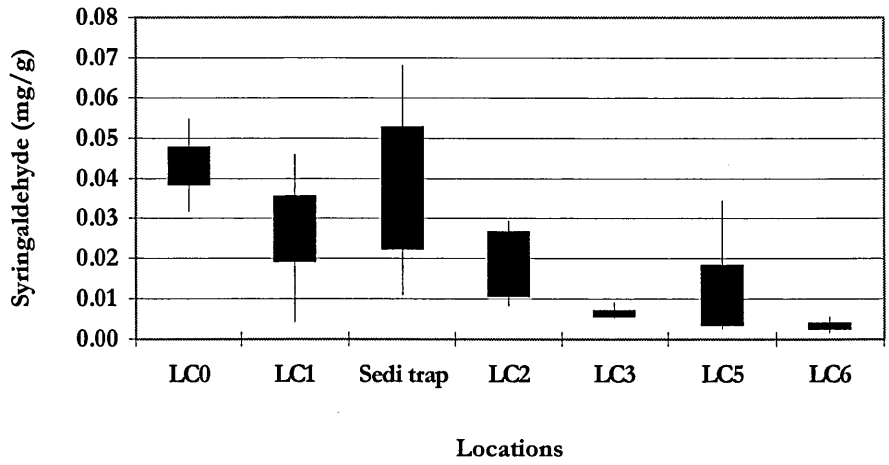
(a) Vanillin.



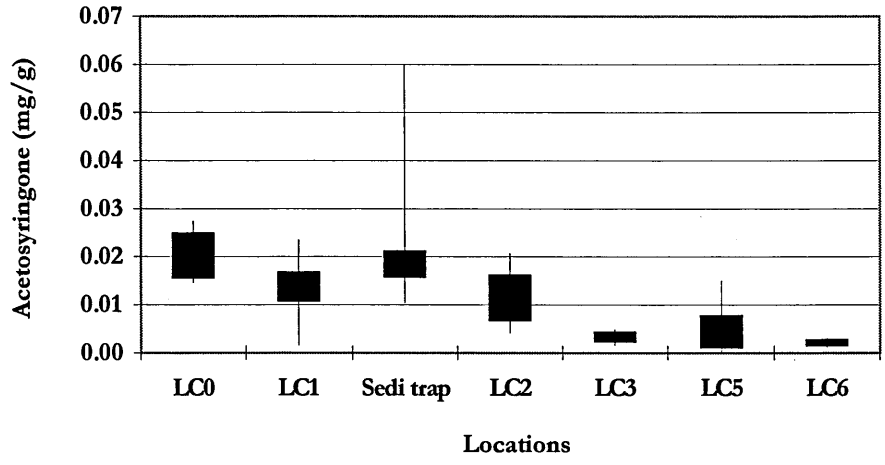
(b) Acetovanillone.



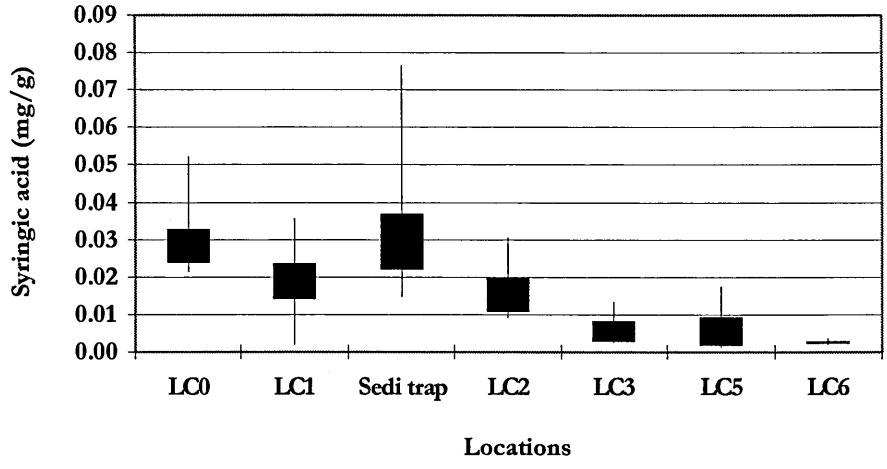
(c) Syringaldehyde.



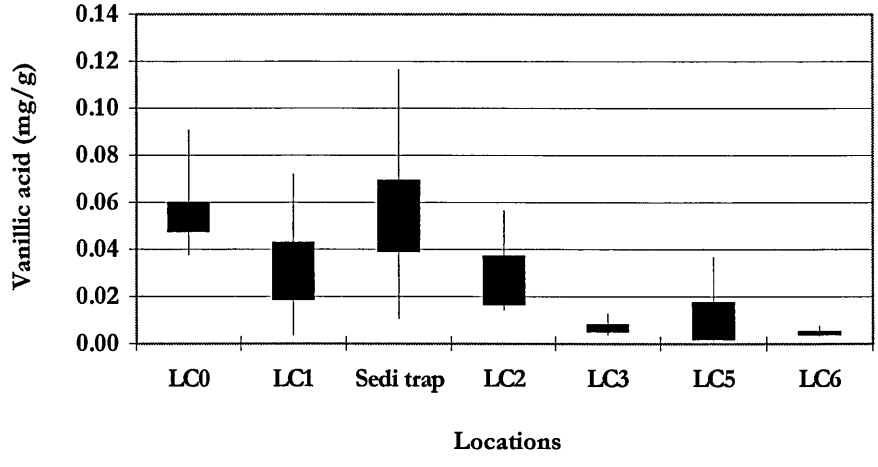
(d) Acetosyringone.



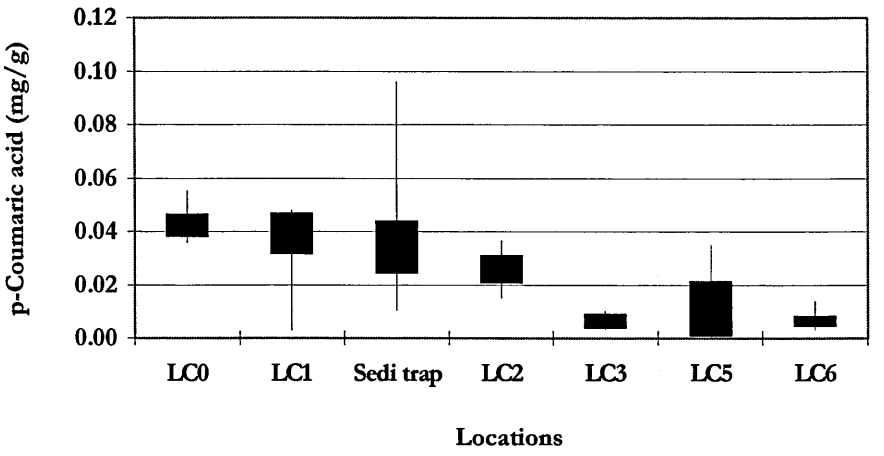
(e) Syringic acid.



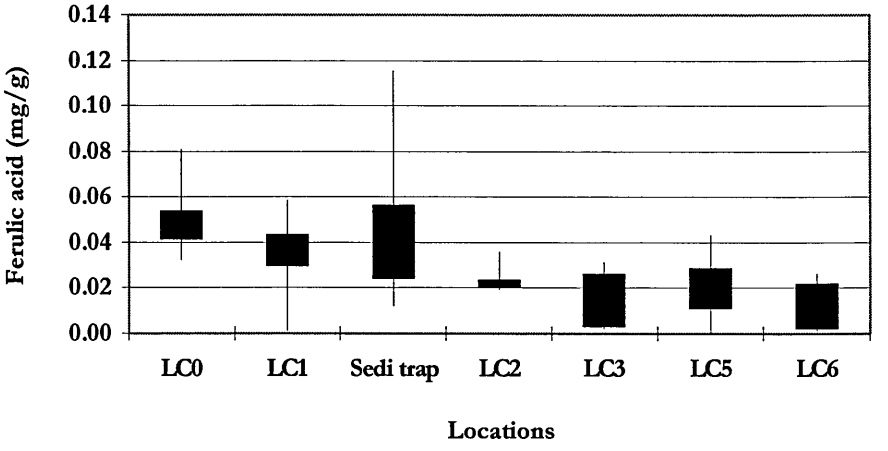
(f) Vanillic acid.



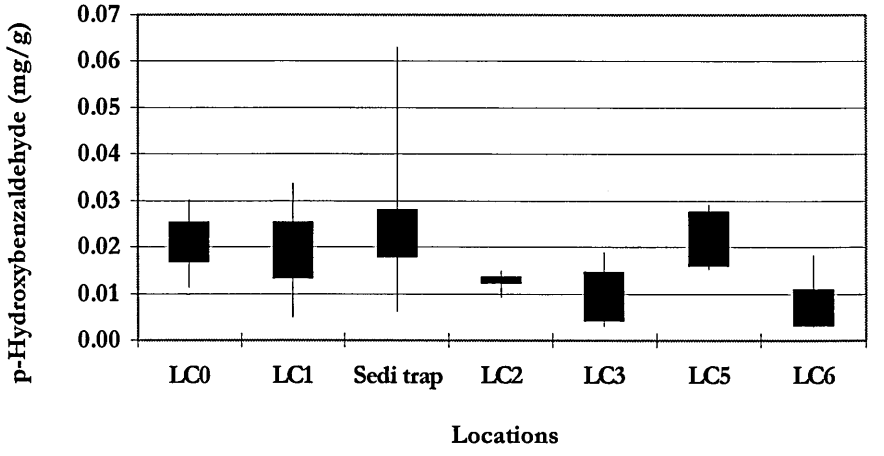
(g) p-Coumaric acid.



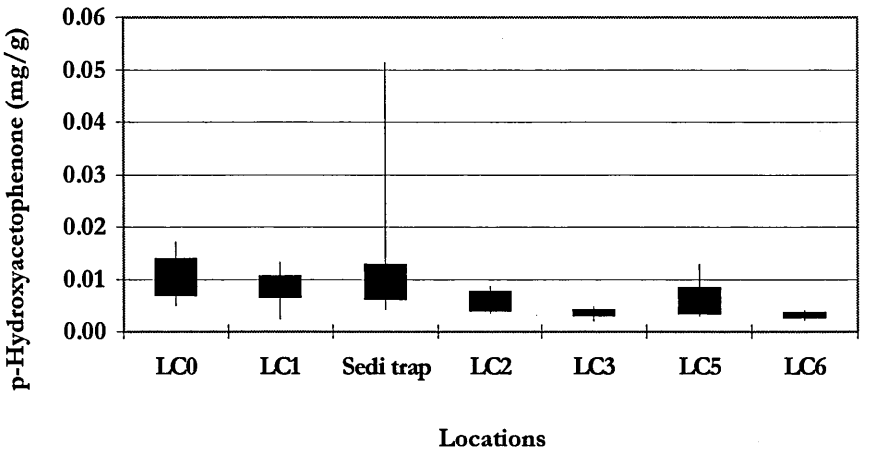
(h) Ferulic acid.



(i) p-Hydroxybenzaldehyde.



(j) p-Hydroxyacetophenone.



(k) p-Hydroxybenzoic acid.

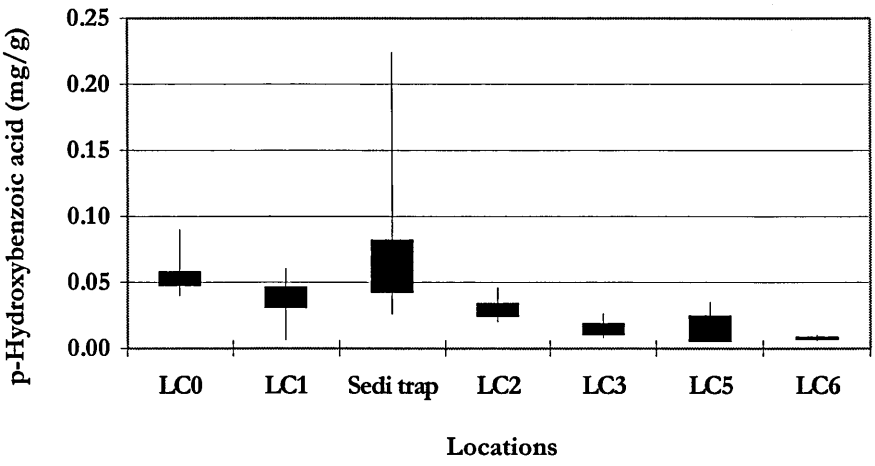


Figure 3.5 Individual lignin phenols in Loch Creran. The box plots are based on the five summary of Min, Q_1, Q_2, Q_3 and Max, in increasing order (see Section 2.2.9 for further details). Note that "sedi trap" means sediment trap.

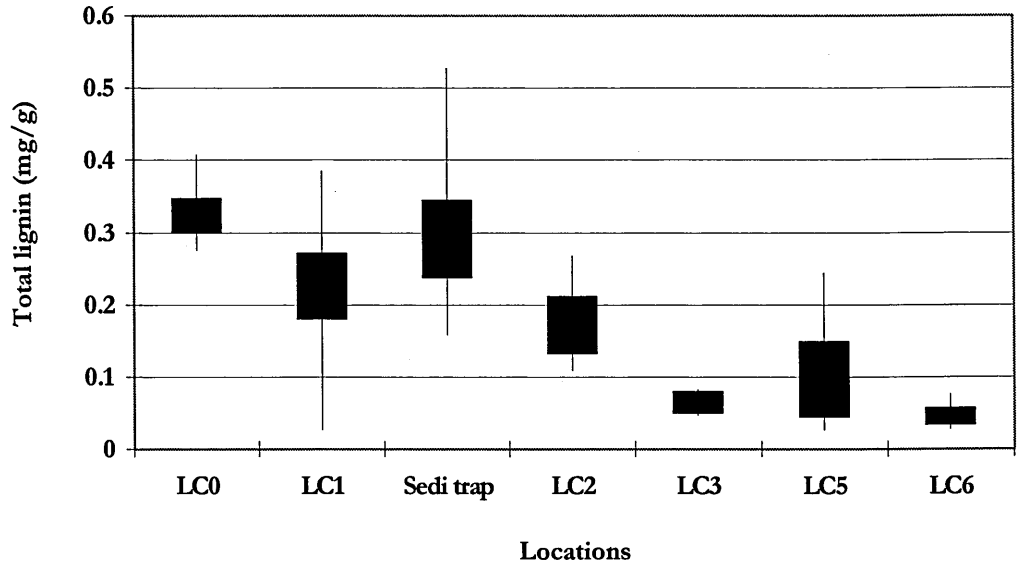
Table 3.5 gives the ranges and mean (± standard deviation) for the lignin parameters. All the P, V, S and C show decrease from the head to the mouth of Loch Creran. LC0 situated nearest the River Creran has the highest abundance for all these lignin phenols and LC6 situated outside the loch has the least abundance for these lignin phenols. At LC0, the mean (mg/g) for these lignin

phenol groups are: V=0.1455, S=0.0917, C=0.0934, and total lignin=0.3305mg/g. At LC6, all these lignin phenols decreased to: V=0.0203, S=0.0078, C=0.0190 and total lignin=0.0470mg/g. The percentages decrease from LC0 to LC6 for these lignin phenols are: V (86.05%), S (91.49%), C (79.66%) and total lignin (85.78%). Overall along the Loch Creran transect, there is significant differences (single factor ANOVA: $p<0.05$) in total lignin across the loch (Figure 3.6), except between LC1 and LC2. This again, suggests the importance of river as the source of terrestrial organic matter input. LC6 situated outside Loch Creran shows significant decrease in total lignin to 0.049mg/g. Overall the (Ad/Al)v values are quite constant throughout the loch, except for occasional huge increase or decrease such as the exceptional high value of 7.6142 for the July 2002 sediment trap sample, and the 0 value (due to zero abundance of vanillic acid) for the LC5 February 2002 sample. There are no significant differences (single factor ANOVA: $p>0.05$) between the sampling locations for the S/V, C/V and (Ad/Al)v ratios.

Table 3.5 Lignin parameters range and mean ± standard deviation for Loch Creran.

Lignin	Lignin parameters range and mean ± standard deviation (in parentheses) for all the locations in Loch Creran.						
	Sediment trap	LC0	LC1	LC2	LC3	LC5	LC6
P (mg/g)	0.06-0.24	0.07-0.09	0.02-0.09	0.04-0.06	0.02-0.04	0.03-0.06	0.01-0.03
V (mg/g)	0.06-0.21 (0.13±0.05)	0.13-0.17 (0.15±0.02)	0.01-0.16 (0.09±0.03)	0.06-0.12 (0.08±0.03)	0.03-0.04 (0.03±0.03)	0.02-0.09 (0.05±0.03)	0.02-0.03 (0.02±0.00)
S (mg/g)	0.05-0.12 (0.09±0.02)	0.08-0.11 (0.09±0.01)	0.009-0.09 (0.06±0.02)	0.03-0.07 (0.05±0.02)	0.01-0.03 (0.02±0.01)	0.004-0.06 (0.02±0.02)	0.006-0.01 (0.01±0.00)
C (mg/g)	0.03-0.17 (0.08±0.04)	0.08-0.11 (0.09±0.01)	0.004-0.10 (0.07±0.02)	0.039-0.06 (0.05±0.01)	0.01-0.04 (0.02±0.02)	0-0.07 (0.03±0.03)	0.01-0.04 (0.02±0.01)
Total lignin (mg/g)	0.16-0.48 (0.30±0.10)	0.30-0.37 (0.33±0.03)	0.03-0.32 (0.22±0.08)	0.12-0.24 (0.18±0.06)	0.05-0.08 (0.07±0.02)	0.03-0.21 (0.10±0.08)	0.03-0.07 (0.05±0.02)
S/V	0.49-1.61 (0.76±0.29)	0.58-0.69 (0.63±0.05)	0.40-1.00 (0.70±0.30)	0.44-0.60 (0.54±0.09)	0.39-0.59 (0.50±0.10)	0.15-0.64 (0.42±0.20)	0.28-0.59 (0.40±0.14)
C/V	0.29-0.95 (0.60±0.18)	0.49-0.85 (0.65±0.14)	0.31-1.01 (0.79±0.22)	0.40-0.89 (0.66±0.24)	0.38-1.47 (0.79±0.59)	0-1.14 (0.66±0.48)	0.37-1.33 (0.88±0.53)
(Ad/Al)v	0.76-7.61 (2.69±1.98)	0.86-1.36 (1.07±0.26)	0.32-1.35 (0.83±0.29)	0.71-1.31 (0.96±0.31)	0.55-1.58 (1.19±0.56)	0-0.99 (0.52±0.41)	0.65-1.12 (0.90±0.20)

(a) Total lignin.



(b) (Ad/Al)v.

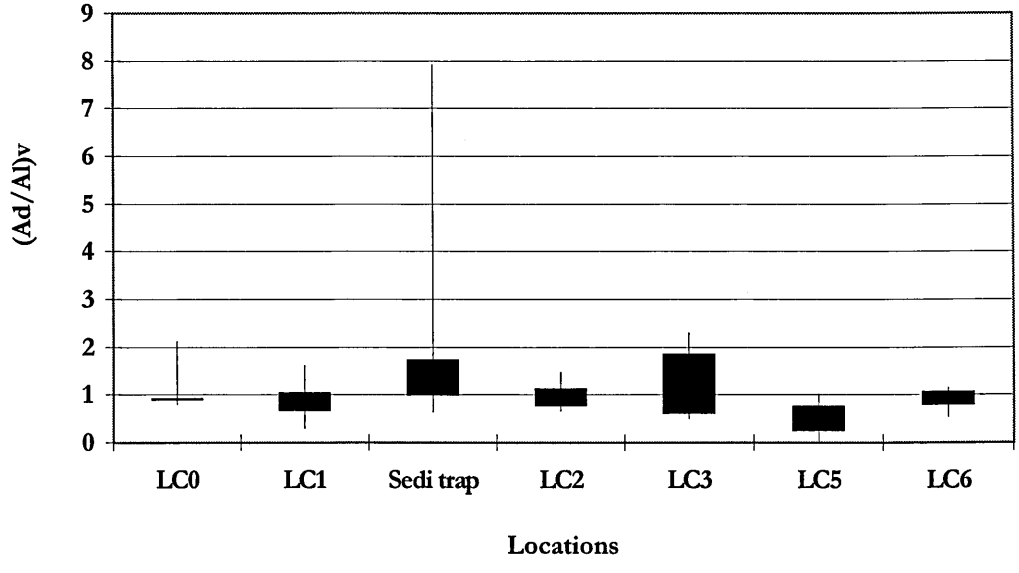


Figure 3.6 Total lignin and (Ad/Al)v for Loch Creran.

3.2.2 Lignin in Loch Etive

The lignin results are given in Table 3.6. Significantly higher or lower values between these lignin parameters between subsequent months are defined using the signs '>' and '<'.

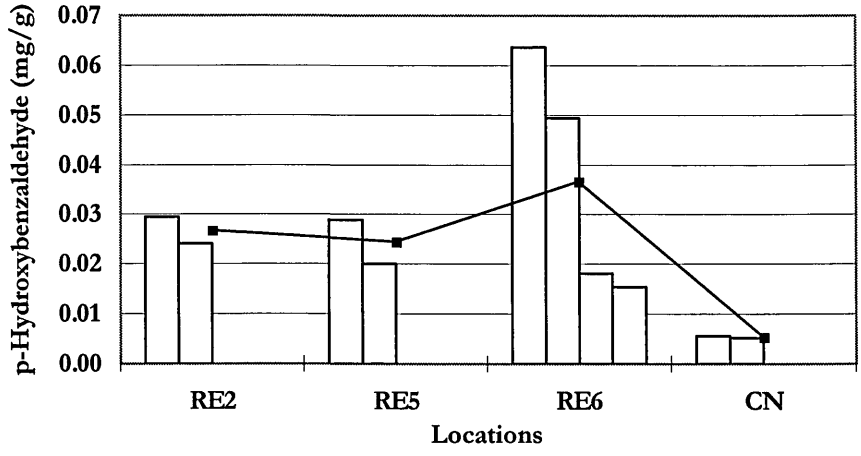
Similarly in Loch Etive, the total lignin decreased from the head to the mouth of the loch, signifying the importance of River Etive as the source of terrestrial organic matter. RE2 has the highest lignin abundance, and Camas Nathais has significantly the lowest lignin abundance. In the uppermost basin, RE2 has a mean total lignin of 0.5477mg/g sediment. Further down the loch RE5 has a mean lignin abundance of 0.4509mg/g and RE6 0.4823mg/g. Total lignin at Camas Nathais decreased to 0.0718mg/g. The (Ad/Al)v value is higher at RE6 (0.8826) than at RE2 (0.7358) and RE5 (0.7195), indicating that lignin becomes more degraded further down the loch. However the (Ad/Al)v is the lowest at Camas Nathais (0.5185), most probably because of the few data collected.

There is no significant difference (single factor ANOVA: $p>0.05$) for the (Ad/Al)v values between all the locations. The total lignin, S, V, C, and the S/V and C/V ratios are mostly significantly (single factor: $p<0.05$) higher at RE2, RE5 and RE6 compared to Camas Nathais. All these indicate the decrease of lignin further down the loch, but the degradation stage of the lignin material remained fairly constant as indicated by the constant (Ad/Al)v values. Hence all the individual lignin phenol shows decrease from the head to the outside of Creran, as shown in Figure 3.7. In Etive, there was slight increase from RE2 to RE6 most probably due to material input into RE6 from River Awe.

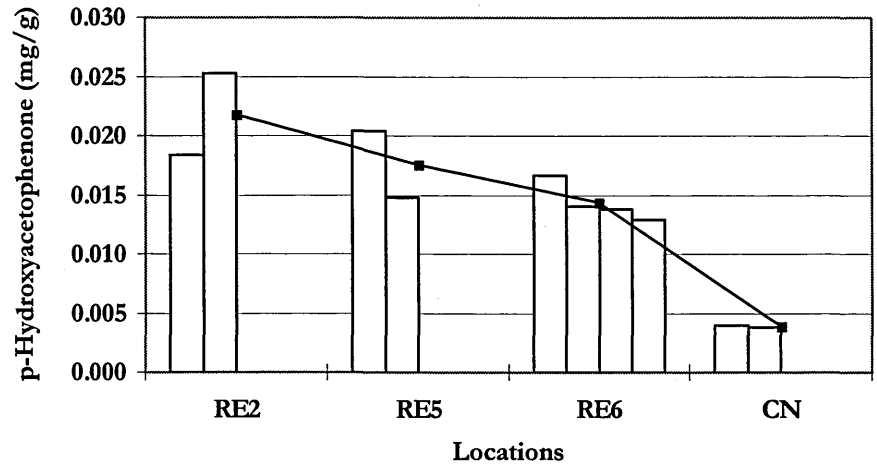
Table 3.6 Lignin parameters for Loch Etive.

Lignin parameters	Lignin concentration (mg/g dry sediment weight) in locations and sampling dates				
	RE2 (14.2.01)	RE5 (17.1.01)	RE6 (14.2.01)	RE6 (20.3.01)	Camas Nathais
(P): p-Hb	0.0268	0.0244	0.0268	0.0263	0.0054
p-Ha	0.0218	0.0176	0.0218	0.0186	0.0039
p-Hba	0.0709	0.0605	0.0709	0.0757	0.0118
(V): Van	0.0670	0.0606	0.0670	0.0548	0.0108
Acetovan	0.0482	0.0310	0.0482	0.0257	0.0093
Va	0.0493	0.0436	0.0493	0.0374	0.0056
(S): Syringyl	0.0573	0.0374	0.0573	0.0341	0.0104
Acetosy	0.1083	0.0969	0.1083	0.0834	0.0034
Sa	0.0036	0.0045	0.0036	0.0032	0.0018
(C): p-Cou	0.0260	0.0297	0.0260	0.0212	0.0021
Fe	0.0685	0.0447	0.0685	0.0322	0.0073
P	0.1195	0.1025	0.1195	0.1206	0.0211
V	0.1645	0.1352	0.1645	0.1179	0.0257
S	0.1692	0.1388	0.1692	0.1207	0.0156
C	0.0945	0.0744	0.0945	0.0534	0.0094
Total lignin	0.5477	0.4509	0.5477	0.4126	0.0718
S/V	1.0286	1.0266	1.0286	1.0237	0.6070
C/V	0.5745	0.5503	0.5745	0.4529	0.3658
(Ad/Al)v	0.7358	0.7195	0.7358	0.6825	0.5185
(Ad/Al)s	0.0628	0.1203	0.0628	0.0938	0.1731

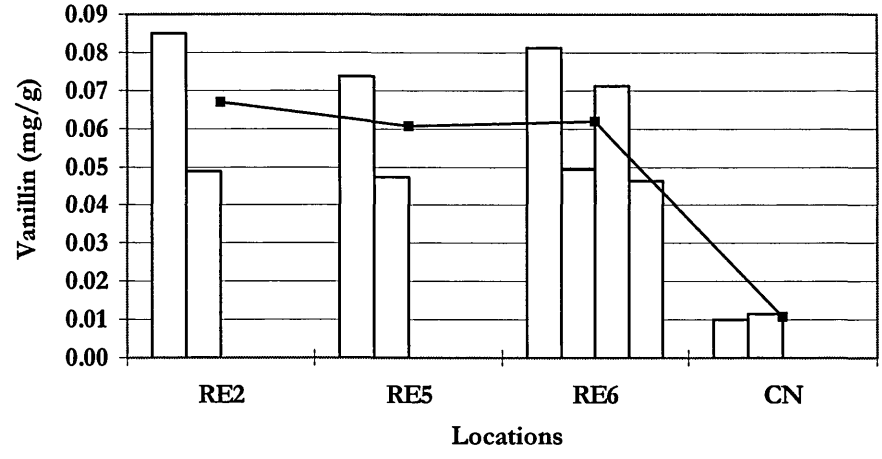
(a) p-Hydroxybenzaldehyde.



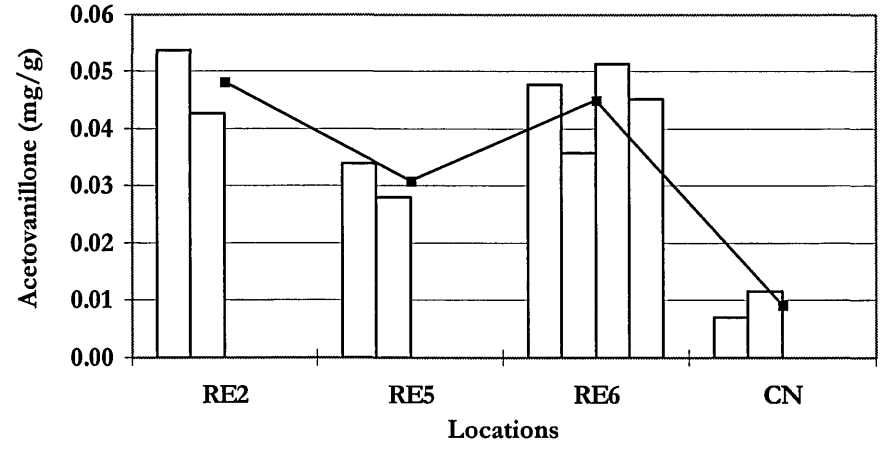
(b) p-Hydroxyacetophenone.



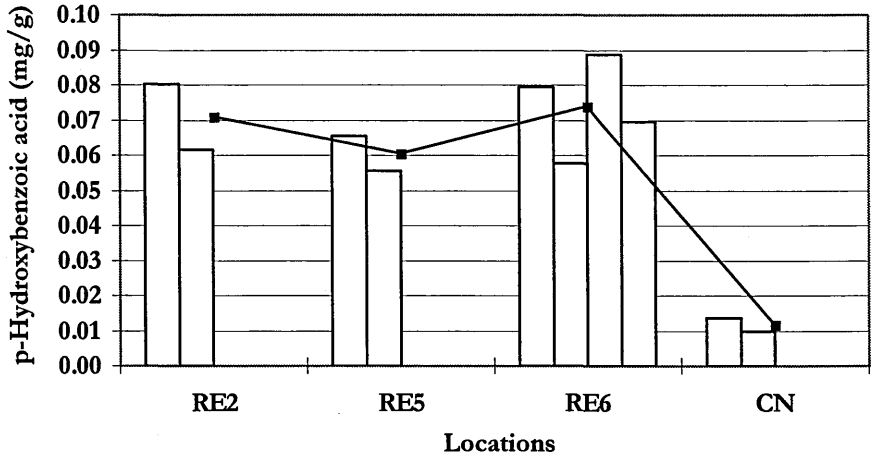
(c) Vanillin.



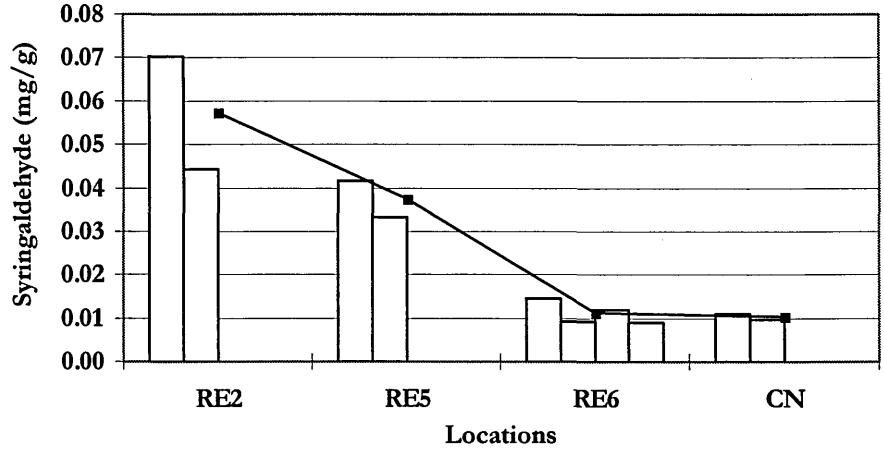
(d) Acetovanillone.



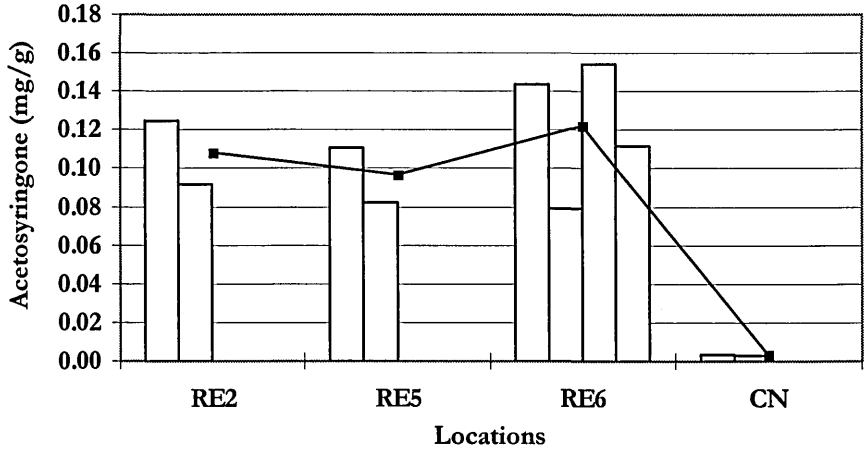
(e) p-Hydroxybenzoic acid.



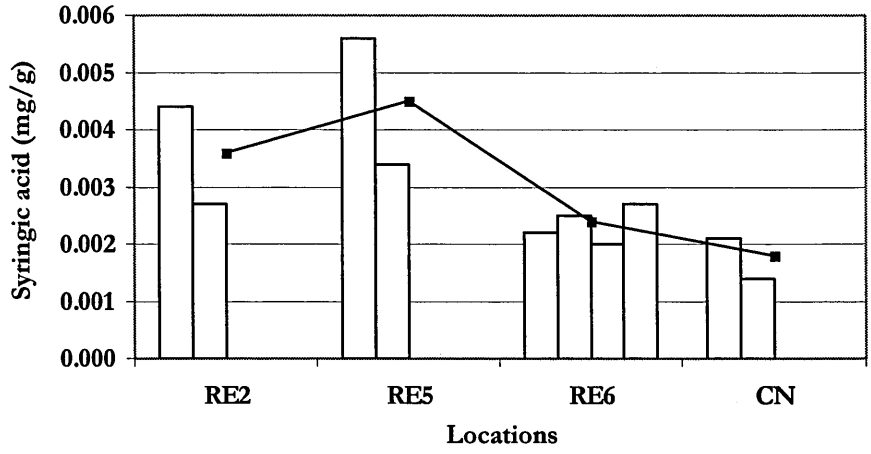
(f) Syringaldehyde.



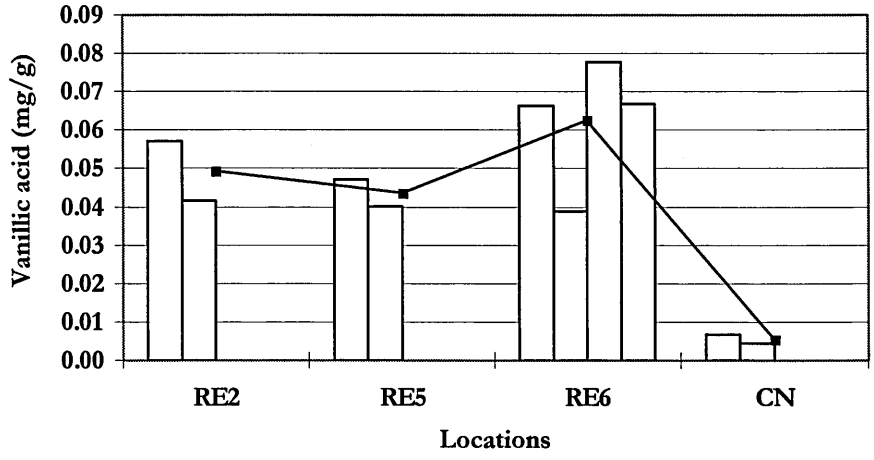
(g) Acetosyringone.



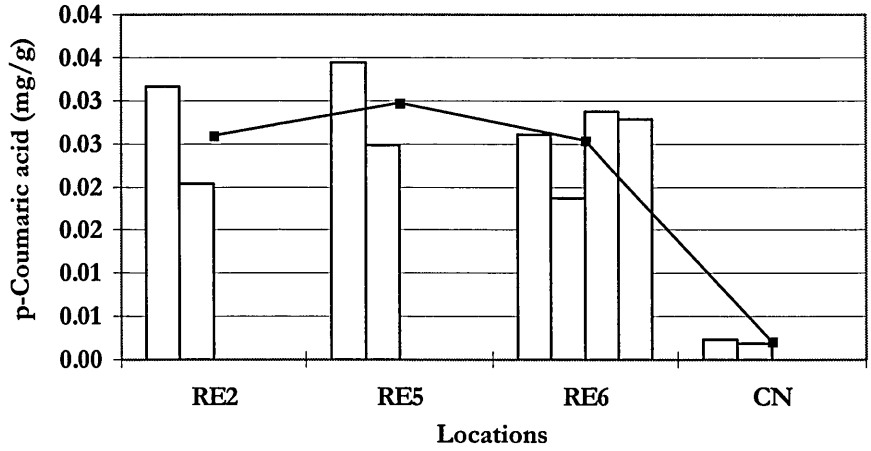
(h) Syringic acid.



(i) Vanillic acid.



(j) p-Coumaric acid.



(k) Ferulic acid.

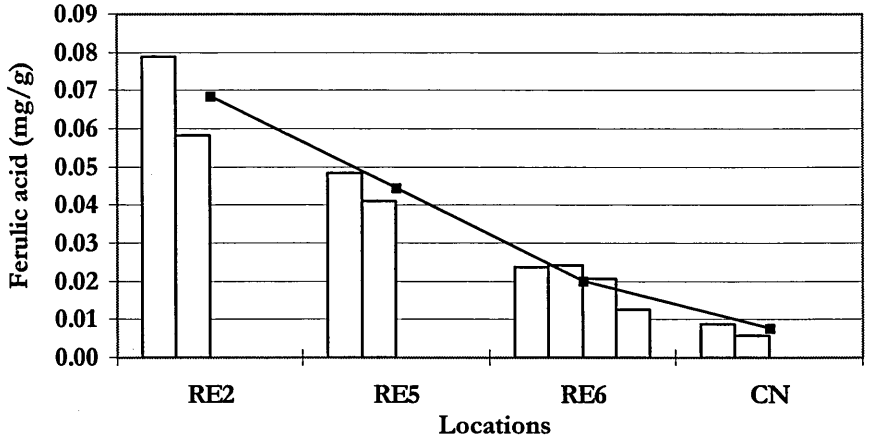


Figure 3.7 Individual lignin phenols in Loch Etive. Each column consists of data from one core, and the line is the mean of the two bars. For example, there are four data points for RE6 because two points represent duplicate analysis for each sampling dates.

3.2.3 Lignin in D/POM

Of all the trials, only one sample of the particulate fraction managed to elucidate lignin phenols. The sample dry weight was 251.07mg, and this was obtained by filtering 2207ml of water sample. The lignin phenols obtained from this 251.07mg particulate fraction were: 0.03mg/g p-hydroxybenzaldehyde, 0.01mg/g vanillin, 0.03mg/g acetovanillone, 0.01mg/g p-hydroxybenzoic acid, 0.17mg/g syringaldehyde, 0.01mg/g syringic acid, 0.39mg/g vanillic acid, and 0.14mg/g p-coumaric acid. These result in S=0.18mg/g, V=0.43mg/g and C=0.14mg/g, and total lignin=0.75mg/g. Hence S/V=0.42 and C/V=0.33.

3.2.4 Summary

1. Lignin and its parameters at individual locations are quite constant with time.
2. The mean ± standard deviation for both lochs Creran and Etive and lignin ranges for transect of Loch Creran (in parentheses) are as follow:
    - Loch Creran:
      Sediment trap: 0.3041±0.0965 mg/g (0.1633-0.4760mg/g)
      LC0: 0.3305±0.0259 (0.2977-0.3671mg/g)
      LC1: 0.2180±0.0765 (0.0284-0.3163mg/g)
      LC2: 0.1760±0.0596 (0.1204-0.2389mg/g)
      LC3: 0.0682±0.0176 (0.0480-0.0799mg/g)
      LC5: 0.1016±0.0801 (0.0330-0.2120mg/g)
      LC6: 0.0470±0.0176 (0.0294-0.0691mg/g)
    - Loch Etive:
      RE2: 0.5477mg/g
      RE5: 0.4509mg/g
      RE6: 0.4823mg/g
      Camas Nathais: 0.0718mg/g
3. Total lignin decreased significantly (single factor ANOVA: $p<0.05$) from the head to the mouth and outside of the lochs. Etive is different from Creran with lateral inputs from River Awe and this affects the pattern of lignin distribution. Hence there is a slight increase of lignin concentration at RE6.
4. There are no significant differences (single factor ANOVA: $p>0.05$) for the C/V and (Ad/Al)v values across Loch Creran. In Loch Etive, no significant difference in (Ad/Al)v was observed.
5. The (Ad/Al)v ranges 0.3201-4.7459 in Loch Creran and 0.5185-1.2296 in Loch Etive.
6. The S/V values range 0.1538-1.6100 in Loch Creran and 0.6070-1.0286 in Loch Etive.
   The C/V values range 0-1.4681 in Loch Creran and 0.2908-0.5745 in Loch Etive.

3.3 OXYGEN UPTAKE FROM INTACT SEDIMENT CORES.

3.3.1 Oxygen uptake rate at Loch Creran

Results for the oxygen uptake tests are given in **Appendix 3**. The mean values are given in Table 3.7. Oxygen uptake rate from intact sediment core is a measure of the oxygen uptake for aerobic organic matter degradation.

Table 3.7 Average oxygen uptake rates for Loch Creran. The symbols ">" and "<" indicate significantly ($p<0.05$) more and less than the value in the following month. Each reading consists of analysis of three cores, and each core triplicate analyses; hence altogether each reading consists of six replicates (see also Appendix 3).

Locations	Oxygen uptake rate (mmol/m^2/day) for 2002										
	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Mean
LC0		18.65>		16.67					15.87		17.06
LC1		14.50<	21.20	18.67<	26.36	27.78>	23.52>	18.87	24.31>	11.84	20.78
LC2		9.23						9.57			9.40
LC3	8.95<	13.58						14.78			12.44
LC5	12.32				14.36<					18.70	15.13
LC6	6.63					14.18>		7.5			9.44

The oxygen uptake rates were measured every month at LC1, but for the other locations, they were visited in successive orders.

Oxygen uptake rate was measured at LC1 on a monthly basis. From data analysis using single factor ANOVA it is found that oxygen uptake rate at LC1 increased significantly ($p<0.05$) from 14.5 mmol/m^2/d in April to 21.2 mmol/m^2/d in May, from 18.67 mmol/m^2/d in June to 26.36 mmol/m^2/d in July 2002, and from 23.52 mmol/m^2/d in September to 24.31 mmol/m^2/d in November 2002. The oxygen uptake rate at LC1 decreased significantly (single factor ANOVA: $p<0.05$) from 27.78 mmol/m^2/d in August to 23.52 mmol/m^2/d in September 2002, and from 24.31 mmol/m^2/d in November to 11.84 mmol/m^2/d in December 2002. The increase of the oxygen uptake rates around the summer from July to September 2002 could be due to increased of phytoplankton production in the summer.

During November and December 2002, the water conditions in the upper most basin of Loch Creran in which both LC1 and LC0 are situated, was very rough. This was most possibly due to strong winds and tides occurring in November and December 2002, and this basin received direct input of freshwater from the River Creran. A slight decrease in oxygen uptake rate was recorded at LC1 in October (18.87 mmol/m^2/d). In November, oxygen uptake rate increased to 24.31 mmole/m^2/d, most probably due to the rough conditions exposing the more labile organic matter to aerobic decomposition. The decrease in the rate to 11.84 mmol/m^2/d in December 2002 could be due the high rate of decomposition of terrestrial materials at LC1 the previous month, resulting in more refractory fraction of materials remaining this month.

The oxygen uptake rate at LC0 was significantly (single factor ANOVA: $p<0.05$) higher during April than June and November 2002. At LC2, there is no significant difference (single factor ANOVA: $p>0.05$) for the oxygen uptake rate between June and November 2002. At LC3, the oxygen uptake rate was significantly lower during March compared to May and September 2002. At LC5, there is significant increase of oxygen uptake rate from July to December 2002. At LC6, there was significant increase of the oxygen uptake rate from March to August and decreased from August to September 2002. At LC2, LC3, LC5 and LC6, the oxygen uptake rates were highest during the later months of the year as detritus from the catchments accumulate. At LC0, the oxygen uptake rate was the highest in April 2002, and decreased towards end of the year, as LC0 is the first location to receive terrestrial input from River Creran during the winter end.

There was no significant difference between LC0 and LC1 (single factor ANOVA: $p>0.05$), but the oxygen uptake rates at LC1 and LC0 are significantly higher than at LC2 (single factor ANOVA: $p<0.05$) as both LC0 and LC1 are situated at the head of the loch. Comparison between LC1 and LC0 for subsequent months saw that oxygen uptake rate was higher at LC1 (24.31 mmol/m^2/d) than LC0 (15.87 mmol/m^2/d) in November 2002. The increase in oxygen uptake at LC1 could be due to the rapid water movement due to plume effect from near the Creran bridge. Oxygen uptake rate at LC1 is significantly higher than all other locations from May to October 2002.

There were, however, significant differences (single factor ANOVA: $p<0.05$) between the individual sampling locations. From Figure 3.8, it is observed that oxygen uptake rates were the highest at LC0 and LC1, most probably due to input of terrestrial organic matter from River Creran into the loch. This was followed by LC5. The higher oxygen uptake rate at LC3 than LC2 was most likely due to the input of marine organic matter at LC3, as lignin abundance was lower at LC3. For further details, see Section 4.5.2.

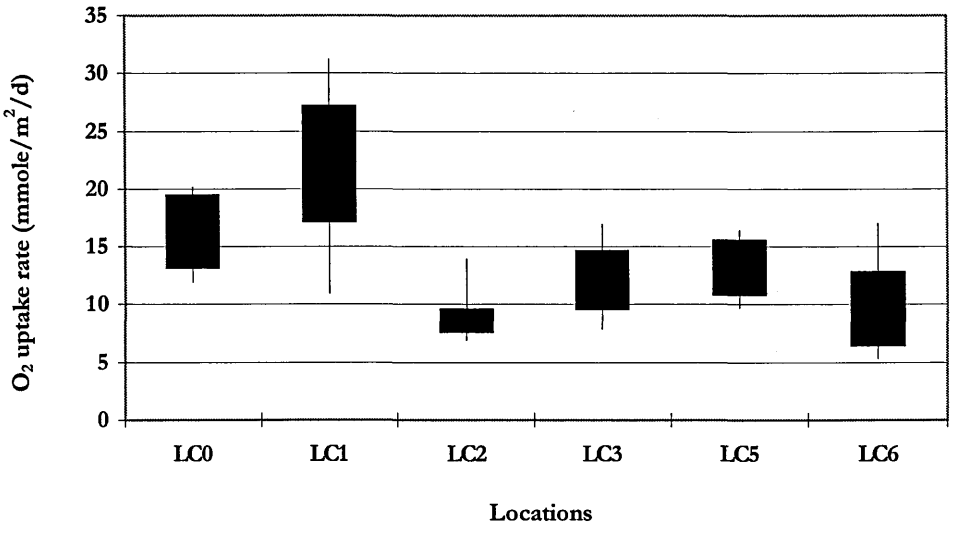


Figure 3.8 Oxygen uptake rates along transect of Loch Creran.

3.3.2 Lignin and the oxygen uptake rate

The results of the linear regression analyses between the oxygen uptake rate with both total lignin and (Ad/Al)v values for different locations are given in Table 3.8. The oxygen uptake rates have significant correlation ($p<0.05$) only with the temperature ($r=0.76$, $r^2=0.58$, $n=9$). These indicate that the oxygen uptake rate increased during summer months, most probably due to more organic matter degradation during the spring phytoplankton bloom in the summer, and decrease in the winter. During the summer the oxygen uptake rates at LC1 have negative correlations with the

total lignin at LC1. The oxygen uptake rates also have significant negative correlations with the (Ad/Al)v values at LC1. Correlation of the oxygen uptake rate with sedimentation shows no significant correlation hence the organic matter degradation at LC1 does not seem to be affected by the organic matter flux.

Table 3.8 Regression analysis between oxygen uptake rate with total lignin and (Ad/Al)v.

Correlation between oxygen uptake rate with the following parameters:	Results from regression analysis:
Wind speed	$r = -0.32$, $r^2=0.11$, $p>0.05$, $n=9$
Temperature	$r=0.76$, $r^2=0.58$, $p<0.05$, $n=9$
Rainfall	$r=0.09$, $r^2=0.01$, $p>0.05$, $n=9$
Sedimentation rate	$r=0.08$, $r^2=0.01$, $p>0.05$, $n=7$
LC1 total lignin	Overall, $r=-0.08$ June to Sep 2002: $r = -0.84$, $r^2=0.11$, $p>0.05$, $n=4$ Sep to Dec 2002: $r=0.74$, $r^2=0.55$, $p>0.05$, $n=5$
LC1 (Ad/Al)v	June to Oct, 2002: $r = -0.93$, $r^2=0.87$, $p<0.05$, $n=5$
Sediment trap total lignin	$r=0.02$, $r^2=0.00$, $p>0.05$, $n=9$
Transect of Loch Creran total lignin	$r=0.58$, $r^2=0.33$, $p>0.05$, $n=6$
Transect of Loch Creran (Ad/Al)v	$r = -0.15$, $r^2=0.02$, $p>0.05$, $n=6$

3.3.3 Summary

1. The oxygen uptake rates in Loch Creran ranged from 6.63 to 27.78 mmole/m^2/day.
2. The oxygen uptake rates at individual locations do not show any consistent change with time.
3. The monthly oxygen uptake rates at LC1 have significant correlation (simple regression analysis: $p<0.05$) with temperatures.
4. Transect-wise of Loch Creran, the oxygen uptake rates decrease significantly from the head to further down the loch.
5. Overall, the oxygen uptake rates do not have significant correlations with the lignin parameters.

3.4 LOSS ON IGNITION

3.4.1 Known compounds

The percentages weight losses due to loss on ignition (LOI) for some known compounds had been by determined by Overnell (*pers.comm.*) (Table 3.9).

Table 3.9 Loss on ignition results for known compounds.

Compound	% labile OM	% refractory OM	% TOM	Rp
Starch	6.38	9.12	15.50	0.59
Cellulose	4.78	6.47	11.25	0.59
Albumin	4.15	7.13	11.28	0.63
Charcoal	0.30	10.95	11.26	0.97
Peat	4.25	2.91	7.17	0.41
Beeswax	9.05	2.85	11.91	0.24

OM=organic matter, TOM=total organic matter.

The Rp value obtained for both starch and cellulose is 0.59. Kristensen (1990) had obtained an Rp index for the egg white as 0.63. Here the Rp index for the albumins is also 0.63. The highest Rp value obtained by Overnell was for the charcoal (0.97); Kristensen (1990) obtained Rp index of 0.64 for humic acid. Plant materials rich in structural carbohydrates generally showed an Rp index around 0.3, whereas Rp for animal tissues rich in proteins is around 0.6 (Kristensen, 1990). The ability to obtain Rp values for known compounds, and the same Rp values obtained by Kristensen (1990) and this study for albumin, confirms the applicability of the Rp index/value to determine the degradation stage of biogenic materials.

3.4.2 Sediment depth profiles

Table 3.10 shows results for the LOI analyses for each layer of the sediment samples. Results for the %TOM are not given in the table, as this is the sum of the % labile and % refractory organic matter. There is constancy for the % labile and % refractory organic matter, and the Rp values between each sediment layer, as seen from the very few significant differences between adjacent sediment layers. Hence in the future only the surface 0-1cm and 9-10 cm layers are analysed. Significant difference in the LOI parameter between each subsequent months is defined as ">" and "<" (significantly higher or lower than).

Table 3.10 Loss on ignition results for sediment depth profiles. Significant differences for the parameter than that in the following month are defined by the significantly higher or lower than signs: ">" and "<".

(a) Loch Etive sediment samples.

Sediment layer (cm)	Sample											
	RE5 (17.1.01)			RE6 (17.1.01)			RE6 (14.2.2001)			RE6 (20.3.2001)		
	%labile	%refrac	Rp	%labile	%refrac	Rp	%labile	%refrac	Rp	%labile	%refrac	Rp
0-1	10.88	9.40	0.46	11.38	7.88	0.41	11.69	4.58 <	0.37	10.88	18.73	0.42
1-2	10.58	8.94	0.43	10.89	8.10	0.43	10.57	8.38	0.44	9.65	18.01	0.46
2-3	10.43	9.14	0.47	10.83	8.90	0.43	10.21	8.15	0.44	10.49	18.10	0.42
3-4	10.49	8.92	0.46	11.68	7.43	0.40	9.64	7.42	0.42	10.99 >	18.12	0.39 <
4-5	10.81	8.68	0.45	10.31	7.14	0.43	9.20	7.82	0.46	9.44	17.28	0.45 <
5-6	10.52	7.74	0.42	10.56	8.20	0.47	8.54	7.87	0.45	8.65	16.70	0.48
6-7	13.02	8.59	0.41	10.97	7.96	0.46	9.34	7.55	0.45	8.46	17.67	0.51
7-8	10.03	8.72	0.48	10.44	7.72	0.44	8.56	7.54	0.44	8.19	16.90	0.50
8-9	9.77	8.93	0.46	10.07	8.53	0.42	7.86	7.84	0.44	7.44	15.66	0.53
9-10	10.24	8.55	0.46	9.35	8.36	0.48	6.95	8.27	0.54	6.72	15.23	0.56

% labile = % labile organic matter, % refrac = % refractory organic matter

(b) Loch Creran sediment samples.

Sediment layer (cm)	Sample								
	LC1 (29.5.2001)			LC1 (29.8.2001)			LC1 (12.12.2001)		
	% labile	% refrac	Rp	% labile	% refrac	Rp	% labile	% refrac	Rp
0-1	11.18	7.67	0.41	4.91	5.58	0.54	11.75	6.70	0.36
1-2	10.86	8.14	0.43	6.66	4.87	0.44	11.19	5.69	0.34
2-3	10.18	7.01	0.41	5.96	4.62	0.46	10.08	5.73	0.36
3-4	10.71	6.59	0.38	6.06	4.27	0.41	9.66	6.15	0.39
4-5	10.69	5.97	0.40	6.94	4.23	0.44	9.33	6.59	0.39
5-6	9.64	7.29	0.44	6.02	4.42	0.48	9.16 >	6.74	0.39
6-7	9.61	6.92	0.44	5.91	4.71	0.44	6.83 <	6.08	0.44
7-8	10.05	7.50	0.43	5.96	4.24	0.42	9.71	6.94	0.40
8-9	9.57	6.71	0.41	5.75	4.04	0.44	8.92	7.33	0.47
9-10	10.88	7.36	0.40	4.76	4.46	0.48	9.24	6.86	0.43

Chapter 3: RESULTS

3.4.3 Sediment samples

The loss on ignition parameters can provide an approximation to the relative abundance of the labile and refractory fractions of the organic matter. The sum of the % labile and % refractory organic matter is considered here to be the % total organic matter (%TOM) although percentage weight loss above 500°C might have been due to the loss of water bound in clay minerals (Howard and Howard, 1990). The Rp index (ratio of the refractory to total organic matter, as explained in section 2.2.5) is used to determine the degradation stage of the organic matter. The loss on ignition (LOI) results consists of means of triplicate analyses (three different cores from the same location). Results for the LOI parameters are shown in Table 3.11. Only LC1 and sediment trap sediments were subjected to monthly analysis.

Table 3.11 Loss on ignition parameters for all sampling locations. Results are mean values of a triplicate analysis, and each replicate consists of one analysis of one sediment layer from one of the three cores in the same layer. The ">" and "<" signs indicate significantly higher or lower than the LOI parameter in the following month.

(a) Sediment trap samples.

Date	% labile OM	% refrac OM	% total OM	Rp
Dec 2001	9.81 <	5.77 <	15.58 <	0.37 <
Jan 2002	11.78 <	8.73	20.51 <	0.43 >
Feb 2002	15.37 >	8.68	24.05 >	0.36 <
Mac 2002	13.08 <	9.58	22.66 <	0.42
April 2002	14.22	10.07	24.29 <	0.41
May 2002	15.71 <	11.18 >	26.89 <	0.42 >
June 2002	20.90 >	7.94	28.84	0.28
July 2002	18.62	8.47	27.09 >	0.31
Aug 2002	17.46	7.87	25.33	0.31
Sep 2002	17.00	8.40	25.40	0.33
Oct 2002	16.12 >	8.86	24.98 >	0.35 <
Nov 2002	11.22	9.09	20.31 >	0.45
Dec 2002	10.42	8.31	18.73	0.44

(b) LC0.

Date	Depth (cm)	% labile OM	% refrac OM	% total OM	Rp
16.7.2001	0-1	9.95	8.76	18.71	0.47
8.1.2002	0-1	9.73	7.41	17.14	0.43
7.3.2002	0-1	9.78	7.07	16.85	0.42
4.4.2002	0-1	9.45	7.01	16.46	0.43
14.11.2002	0-1	10.25	6.15	16.40	0.38
	9-10	8.78	4.83	13.61	0.36

(c) LC1.

Date	Depth (cm)	% labile OM	% refrac OM	% total OM	Rp
29.5.2001	0-1	11.18	7.67	18.85	0.41
	9-10	10.88	7.36	18.24	0.40
2.7.2001	0-1	10.48	8.29	18.77	0.44 >
16.7.2001	0-1	10.92 >	7.81 >	18.73 >	0.42 <
29.8.2001	0-1	4.91	5.58 >	9.87	0.54
	9-10	4.76	4.46	9.22	0.48
28.9.2001	0-1	5.25	4.65 <	9.90 <	0.47 >
24.10.2001	0-1	10.93	6.82	17.75	0.39
12.12.2001	0-1	11.75 >	6.70	18.45	0.36 <
	9-10	9.24	6.86	16.10	0.43
8.1.2002	0-1	8.88 <	7.11	15.99 >	0.44
6.2.2002	0-1	7.33 <	6.21	13.54 <	0.46
7.3.2002	0-1	10.65	7.34	17.99	0.41
	9-10	10.39	6.75	17.14	0.39
4.4.2002	0-1	9.03	6.53 <	15.56	0.42 <
7.5.2002	0-1	6.65 <	8.84	15.49	0.57 >
4.6.2002	0-1	9.24 >	8.16 >	17.40 >	0.47 >
2.7.2002	0-1	2.96 <	0.69 <	3.65 <	0.19 <
1.8.2002	0-1	12.09	7.86	19.94	0.39
2.9.2002	0-1	12.70	7.84	20.54	0.38
	9-10	12.45	6.57	19.02	0.35
30.9.2002	0-1	11.56	6.80	18.36	0.37
14.11.2002	0-1	10.49	7.30 <	17.80	0.41
12.12.2002	0-1	10.41	8.56	18.97	0.45
	9-10	9.18	7.40	16.58	0.45

(d) LC2.

Date	Depth (cm)	% labile OM	% refrac OM	% total OM	Rp
29.5.2001	0-1	5.99 <	6.25	12.24 <	0.51 >
24.10.2001	0-1	7.94 >	5.69	13.63	0.42
7.3.2002	0-1	6.13	6.46	12.59	0.51
4.4.2002	0-1	6.46	6.00	12.46	0.48
2.9.2002	0-1	6.54	6.34	12.88	0.49

(e) LC3.

Date	Depth (cm)	% labile OM	% refrac OM	% total OM	Rp
2.7.2001	0-1	4.26	5.48	9.74	0.53
12.12.2001	0-1	2.76	4.34	7.10	0.61
21.3.2002	0-1	2.58	3.78	6.36	0.54
7.5.2002	0-1	2.97	4.95	7.92 <	0.63
30.9.2002	0-1	4.77	6.02	10.79 >	0.62
	9-10	3.77	4.64	8.41	0.58

(f) LC5.

Date	Depth (cm)	% labile OM	% refrac OM	% total OM	Rp
29.8.2001	0-1	0.86	1.39	2.25	0.62
6.2.2002	0-1	0.70 <	1.99	2.68	0.67 >
21.3.2002	0-1	1.92 <	1.88 <	3.80 <	0.50 >
2.7.2002	0-1	12.11 >	7.64 >	19.74 >	0.39 <
12.12.2002	0-1	1.58	2.57	4.16	0.62
	9-10	1.65	1.72	3.37	0.51

(g) LC6.

Date	Depth (cm)	% labile OM	% refrac OM	% total OM	Rp
28.9.2001	0-1	3.28	4.96	8.24	0.60 <
21.3.2002	0-1	3.48	6.22	9.69	0.68
1.8.2002	0-1	4.05	6.91	10.96	0.63

(h) Loch Etive.

Location	Date	Depth (cm)	% labile OM	% refrac OM	% total OM	Rp
RE6	17.1.2001	0-1cm	11.38	7.88	19.26	0.41
		9-10cm	9.35	8.36	19.19	0.48
	14.2.2001	0-1cm	12.77 >	6.75< (18.73)	19.52 >	0.34 <
		9-10cm	6.95	8.27	15.22	0.54
	20.3.2001	0-1cm	10.88 >	18.73 >	29.62 >	0.42 <
		9-10cm	6.72	15.23	21.96	0.56
RE2	14.2.2001	0-1cm	10.56	7.13	17.69	0.40
RE5	17.1.2001	0-1cm	10.88 >	9.40	20.28 >	0.46
		9-10cm	10.24	8.55	18.79	0.46
Camas Nathais	20.3.2001	0-1cm	3.04	12.08	15.11	0.75

For the sediment trap samples, the ratio of the % labile to the % refractory organic matter increases, hence the Rp values decreased from June to October 2002, indicating the presence of the more labile fraction of the organic matter during summer. For the LC1 sediments, there is no consistent trend in the increase or decrease of the LOI parameters. Similar trends of consistencies are observed in all other individual locations.

As there are mostly no significant differences in the LOI parameters in individual sampling locations, these are averaged and the results tabulated in Table 3.12. One interesting observation is that whilst the percentage organic matter decreases, the Rp values increased further down the lochs. The sediment trap samples have the highest % labile organic matter and lowest Rp value, indicating the presence of more labile organic matter. The Rp value is slightly lower at LC5 than LC3, indicating the input of fresher terrestrial materials into LC5. At LC6, % refractory organic matter and Rp values are higher than at LC3 and LC5, indicating that there is more refractory

organic matter remaining at LC6. From LC0 to LC6, the percentages labile, refractory, and total organic matter all decreased 63.38%, 17.17% and 43.72%, but the Rp value increased 48.84%. Similarly for Loch Etive, the LOI parameters decreased from the head to outside the loch. Camas Nathais has the highest % refractory organic matter and hence highest Rp index compared to all other locations in Loch Etive. From RE5 to Camas Nathais the percentages labile and total organic matter decreased 72.06% and 25.49%, but the % refractory organic matter and Rp value increased 28.51% and 63.04%.

There are no significant (single factor ANOVA: $p>0.05$) differences in the LOI parameters between LC0 and LC1, indicating that these two locations have almost the same organic matter content. The LOI parameters at LC0 and LC1 are significantly higher (single factor ANOVA: $p<0.05$) than sediments from other locations. At LC2, the % labile, refractory and total organic matter are significantly higher than at LC3. However the Rp value at LC3 is significantly higher than those obtained from LC0, LC1 and LC2 indicating that further down the loch, there is a greater refractory fraction of the organic matter, as the labile fractions have undergone degradation.

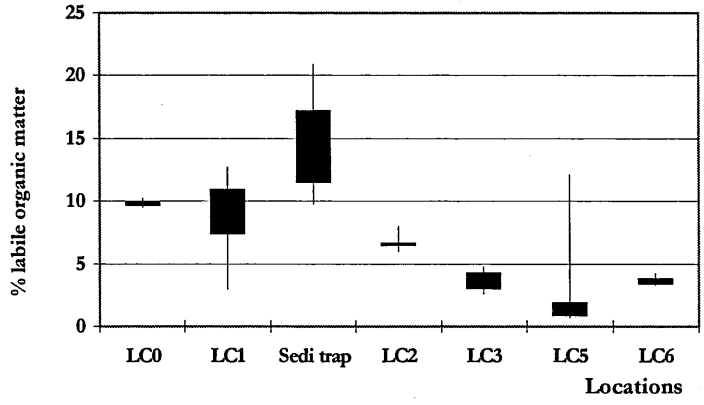
Table 3.12 Loss on ignition parameters for lochs Creran and Etive transects.

Site	% labile	% refrac	% total OM	Rp
LC0	9.83	7.28	17.11	0.43
Sediment trap	14.75	8.69	23.44	0.38
LC1	9.34	6.88	16.19	0.42
LC2	6.61	6.15	12.76	0.48
LC3	3.47	4.91	8.38	0.59
LC5	3.43	3.09	6.53	0.56
LC6	3.60	6.03	9.63	0.64
RE2	10.56	7.13	17.69	0.40
RE5	10.88	9.40	20.28	0.46
RE6	11.68	11.12	22.80	0.39
Camas Nathais	3.04	12.08	15.11	0.75

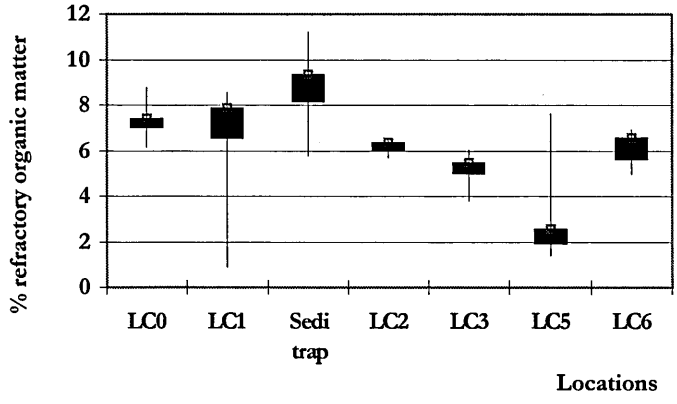
The box plots (Figure 3.9) show that the % labile, % refractory and % total organic matter are highest at the uppermost basin in the sediment trap, LC0 and LC1 sediments, and decrease down the loch. The Rp values however, increase down the loch. For Loch Etive, the slight increases in

the % labile, % refractory and % total organic matter, and the decrease of Rp value at RE6 indicate the input of fresher new materials from River Awe.

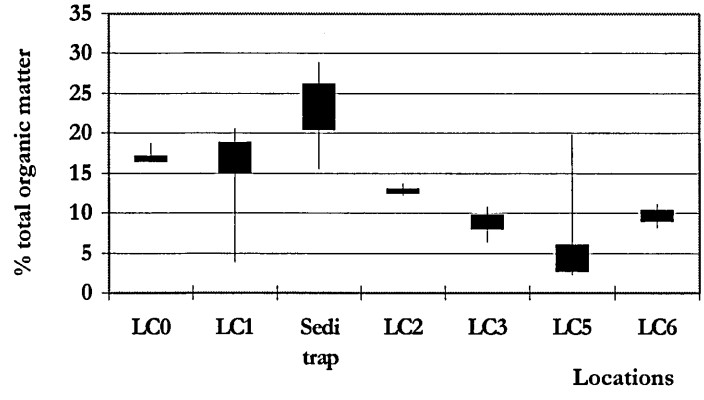
(a) % labile organic matter.



(b) % refractory organic matter.



(c) % total organic matter.



(d) Rp values.

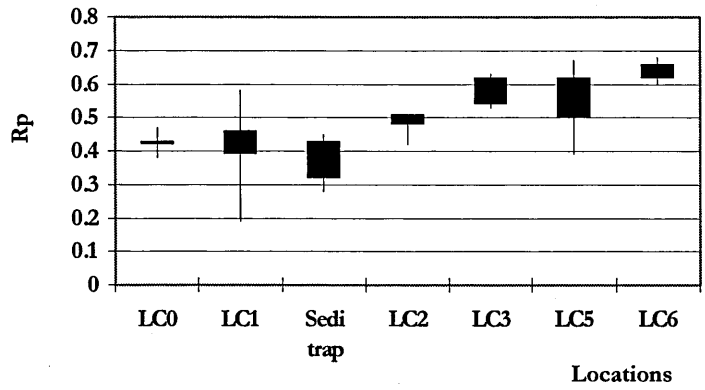


Figure 3.9 Box plot of the LOI parameters for Loch Creran.

3.4.4 Lignin and loss on ignition

The results of the regression analyses between total lignin with % labile, % refractory and % total organic matter, and between (Ad/Al)v and Rp value are tabulated in Table 3.13.

There are significant correlations between total lignin versus % labile organic matter and between total lignin versus the Rp values along transects of both Lochs Creran and Etive. Further down the lochs, the total lignin and % labile organic matter decreased indicating decrease in the terrestrial organic matter content and the Rp values increase indicating that these materials had become more degraded.

Table 3.13 Regression analyses between total lignin and loss on ignition parameters.

Location	Correlation between:	With LOI parameters:	Results for regression analysis:
LC1	Lignin	% labile OM	r=0.41, r^2=0.16, p>0.05, n=9
		% refractory OM	r=0.59, r^2=0.35, p>0.05, n=9
		% TOM	r=0.5, r^2=0.28, p>0.05, n=9
		Rp	r=-0.31, r^2=0.09, p>0.05, n=11
	(Ad/Al)v	Rp	r= -0.06, r^2=0.00, p>0.05, n=9
Sediment trap	Lignin	% labile OM	r= -0.23, r^2=0.06, p>0.05, n=13
		% refractory OM	r= -0.06, r^2=0.00, p>0.05, n=13
		% TOM	r= -0.23, r^2=0.05, p>0.05, n=13
		Rp	r=0.13, r^2=0.02, p>0.05, n=13
	(Ad/Al)v	Rp	r= -0.29, r^2=0.08, p>0.05, n=13
Transect of Loch Creran	Lignin	% labile OM	r=0.93, r^2=0.87, p<0.05, n=6
		% refractory OM	r=0.39, r^2=0.15, p>0.05, n=6
		% TOM	r=0.83, r^2=0.68, p<0.05, n=6
		Rp	r=-0.95, r^2=0.90, p<0.05, n=6
	(Ad/Al)v	Rp	r= -0.05, r^2=0.00. p>0.05, n=6
Transect of Loch Etive	Lignin	% labile OM	r=0.97, r^2=0.93, p<0.05, n=4
		% refractory OM	r=-0.75, r^2=0.56, p>0.05, n=4
		% TOM	r=0.68, r^2=0.47, p>0.05, n=4
		Rp	r=-0.99, r^2=0.98, p<0.05, n=4
	(Ad/Al)v	Rp	r=-0.92, r^2=0.84, p>0.05, n=4

For the LC1 samples, the data from 7.5.2002 and 2.7.2002 are omitted in these regression analyses as the total lignin in 7.5.2002, and LOI parameters obtained from 2.7.2002 were hugely lower than normally obtained.

3.4.5 Summary

1. The LOI parameters from individual locations remain constant with time.
2. The LOI parameters are also constant between the 0-1cm and 9-10cm sediment layer.
3. However, the % labile organic matter seems to increase and Rp values decrease in the summer for the sediment trap samples.
4. Transect-wise of both lochs, the % labile, % refractory and % total organic matter decrease while the Rp values increase from the head towards the mouth of the lochs.
5. Total lignin has significant (regression analysis: p<0.05) correlation with the % labile (r=0.93) and % total organic matter (r=0.83), and Rp value (r=-0.95) in Loch Creran transect.
6. Total lignin has significant (p<0.05) correlation with the % labile organic matter (r=0.97) and Rp value (r=-0.99) in Loch Etive transect.

3.5 STABLE CARBON ISOTOPES

The sedimentary stable carbon isotopes ratios are useful to determine the relative abundances between the terrestrial and marine organic matter. A more negative $\delta^{13}C$ value indicates a relatively higher abundance of terrestrial organic matter whilst a higher $\delta^{13}C$ value indicates a relatively higher abundance of marine organic matter.

3.5.1 Known Compounds

The $\delta^{13}C$ values of some known compounds were firstly determined (Table 3.14a), in order to confirm the validity of this experiment to distinguish between the terrestrial and marine organic matter. Marine organisms such as algae have higher $\delta^{13}C$ values. The mean $\delta^{13}C$ values obtained for the lignin hydrolytic is –14.28‰, indicating that this substance was made from C4 plant sources, as also reported by Hedges and Parker (1976) and Onstad *et al.* (2000). Hedges and Parker (1976) found that both *Spartina alterniflora* and *Canavalia maritima* have $\delta^{13}C$ values near –11‰, a characteristic of C4 plants and that the marine alga *Sargassum* has a $\delta^{13}C$ value near –15‰, a trend for marine phytoplankton from warm waters. Here the marine green alga *Tetracelmis chui** gave a mean $\delta^{13}C$ value of -17.17‰ and the marine brown alga *Pavlova lutheri* gave a mean $\delta^{13}C$ value of –22.08‰ (Catalogue of the UK National Culture Collection, UKNCC).

Analysis for three individual lignin phenols standard: vanillin, syringaldehyde and syringic acid, gave more depleted $\delta^{13}C$ values. Syringic acid has the most depleted $\delta^{13}C$ value of -32.59‰. Syringaldehyde and vanillin both have $\delta^{13}C$ values of -27.46 and -27.23‰. Plant materials gave more depleted $\delta^{13}C$ value from –24.81‰ to –30.55‰. Bracken and birch leaves gave more

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* ***Tetraselmis chui***: CCAP 8/6; was isolated by Pringsheim from material collected by Chui at Millport, Isle of Cumbrae, Scotland.

Pavlova lutheri: CCAP 931/1; was isolated by Michael Droop in June 1953 from a small intertidal pool near Milport, Isle of Cumbrae, Scotland (Catalogue of the UK National Culture Collection, UKNCC).

depleted $\delta^{13}C$ values compared to their stems. The most depleted $\delta^{13}C$ values are observed for spruce needles.

3.5.2 Sediment samples

The $\delta^{13}C$ values for the sediment trap samples are quite constant (Figure 3.14b), with the mean value of –21.33 ± 0.23‰, and the average coefficient of variation 1.08%. From data analysis using single-factor ANOVA, significant differences ($p<0.05$) for the trap samples occur between these two subsequent months: December 2001 (-24.66‰) and January 2002, January and February 2002, and February and March 2002. In April, May and June the $\delta^{13}C$ values increased to –19‰, indicating the presence of marine organic matter from the spring phytoplankton bloom.

There is no significant difference in the $\delta^{13}C$ values between the surface 0-1cm and bottom 9-10cm sediment layers for all sampling locations, except that from LC1 on the 2.9.2002. There is mostly no significant difference between lignin parameter at individual locations with time. Among all the different sampling time there is only significant difference of $\delta^{13}C$ values for LC1 between the September and December 2002.

Transect-wise of Loch Creran the $\delta^{13}C$ values for Creran head and LC0 sediments both show the most depleted values of – 24.45‰ and –24.64‰, indicating the presence of terrestrial organic matter. LC1 has a $\delta^{13}C$ values of –23.57‰ and for the sediment trap samples –21.33‰. The $\delta^{13}C$ values become more enriched further down the loch. $\delta^{13}C$ values from LC3, LC5 and LC6 showed the presence of marine organic matter: -16.79‰, -18.99‰ and –14.62‰, respectively. LC6 situated outside the loch has the highest $\delta^{13}C$. RE2, RE5 and RE6 in Loch Etive have the same $\delta^{13}C$ values of around –25.70‰, indicating the presence of terrestrial organic matter within the loch. Camas Nathais has significantly the highest $\delta^{13}C$ values of –11.25‰, indicating the presence of marine organic matter. Hence the $\delta^{13}C$ values in the upper lochs sediments are more depleted than sediments from further down and outside the lochs.

Table 3.14 Stable carbon isotopes results. Mean values are obtained from replicate analysis of samples from the same sampling location, but different cores. Reproducibility is calculated as the coefficient of variation (CV).

(a) $\delta^{13}C$ values for known compounds.

Component	Rep	$\delta^{13}C$	Mean	± Probable uncertainty	% uncertainty
Lignin hydrolytic	1	-14.46	-14.28	± 0.13	0.91
	2	-14.10			
Vanillin	1	-27.26	-27.23	± 0.02	0.08
	2	-27.20			
Syringaldehyde	1	-27.45	-27.46	± 0.01	0.03
	2	-27.47			
Syringic acid	1	-32.46	-32.59	± 0.09	0.28
	2	-32.71			
Bracken leaves	1	-27.62	-27.35	± 0.20	0.73
	2	-27.07			
Bracken stems	1	-24.95	-24.81	± 0.10	0.40
	2	-24.67			
Birch leaves	1	-30.45	-29.75	± 0.50	1.68
	2	-29.05			
Birch stems	1	-27.57	-27.73	± 0.11	0.40
	2	-27.88			
Spruce needles	1	-30.72	-30.55	± 0.13	0.43
	2	-30.37			
Green algae	1	-16.78	-17.17	± 0.28	1.63
	2	-17.56			
Brown algae	1	-21.96	-22.08	± 0.09	0.38
	2	-22.19			

The green alga is *Tetraselmis chui* and the brown alga is *Pavlova lutheri*. This lignin hydrolytic is a substance used as part of the method validation process.

(b) $\delta^{13}C$ values for sediment trap samples.

Date	Replicates	$\delta^{13}C$ (‰)	Mean	± Probable uncertainty	% uncertainty
Dec,01	1	-24.35	-24.66	± 0.22	0.89
	2	-24.96			
Jan,02	1	-21.99	-21.94	± 0.04	0.18
	2	-21.89			
Feb,02	1	-23.55	-23.57	± 0.01	0.04
	2	-23.58			
Mac,02	1	-22.37	-22.48	± 0.08	0.36
	2	-22.58			
April,02	1	-20.01	-19.65	± 0.25	1.27
	2	-19.29			
May,02	1	-19.58	-19.58		
June,02	1	-20.01	-19.80	± 0.16	0.81
	2	-19.58			
July,02	1	-20.89	-21.21	± 0.23	1.08
	2	-21.53			
Aug,02	1	-20.47	-20.54	± 0.05	0.24
	2	-20.61			
Sep,02	1	-20.42	-20.78	± 0.25	1.20
	2	-21.14			
Oct,02	1	-20.17	-20.70	± 0.37	1.79
	2	-21.22			
Nov,02	1	-21.01	-21.03	± 0.01	0.05
	2	-21.05			
Dec,02	1	-21.62	-21.37	± 0.18	0.84
	2	-21.11			

(c) $\delta^{13}C$ for sediments from Loch Creran.

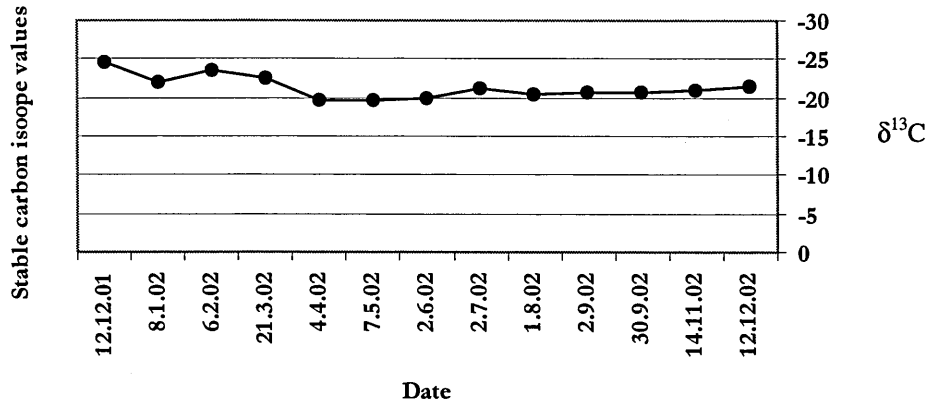
Locations	Dates	Depth	Mean	Probable uncertainty	% uncertainty
Creran head	5.12.2001	0-1cm	-25.04	±0.03	0.11
		9-10cm	-23.86	±0.76	3.20
LC0	4.6.2002	0-1cm	-25.21	±0.04	0.14
	14.11.2002	0-1cm	-24.45	±0.23	0.95
		9-10cm	-24.28	±0.09	0.38
LC1	4.6.2002	0-1cm	-23.13	±0.45	1.94
		9-10cm			
	2.9.2002	0-1cm	-23.20	±0.08	0.34
		9-10cm	-24.33	±0.30	1.25
	12.12.2002	0-1cm	-23.98	±0.17	0.71
		9-10cm	-24.57	±0.29	1.18
LC2	4.4.2002	0-1cm	-24.02	±0.17	0.71
	7.5.2002	0-1cm	-23.96	±0.27	1.12
		9-10cm	-23.37	±0.24	1.03
LC3	7.5.2002	0-1cm	-16.10	±0.92	5.71
	30.9.2002	0-1cm	-17.07	±0.70	4.10
		9-10cm	-18.59	±0.08	0.42
LC5	2.7.2002	0-1cm	-20.96	±3.54	16.87
	12.12.2002	0-1cm			
		9-10cm	-17.14	±1.65	9.62
LC6	1.8.2002	0-1cm	-15.18	±0.43	2.84
	30.9.2002	0-1cm	-14.38	±0.52	3.59
		9-10cm	-14.30	±0.04	0.25

(d) $\delta^{13}C$ for sediments from Loch Etive.

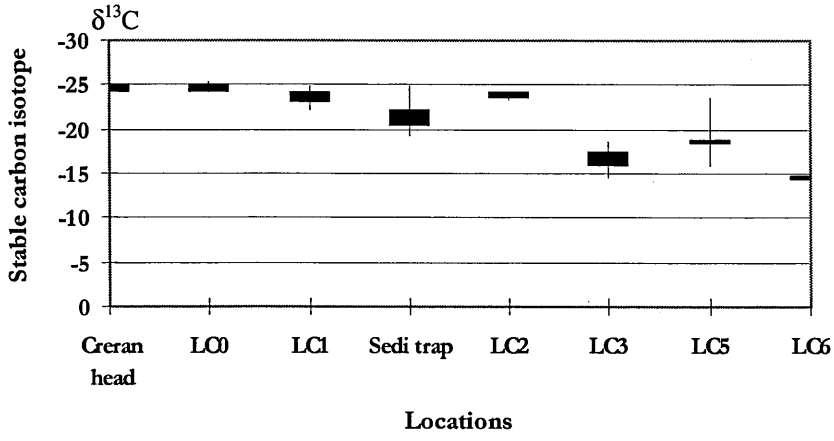
Locations	Date	Rep	$\delta^{13}C$ (‰)	Mean	± Probable uncertainty	% uncertainty
RE6	14.2.2001	1	-25.80	-25.71	±0.06	0.23
		2	-25.62			
RE5	17.1.2001	1	-26.20	-25.75	±0.33	1.28
		2	-25.29			
RE2	14.2.2001	1	-25.91	-25.76	±0.11	0.43
		2	-25.60			
Camas Nathais		1	-13.47	-11.25	±1.57	13.96
		2	-9.03			

The extent to which terrestrial materials contributed to the sediment organic matter is studied by plotting the $\delta^{13}C$ values across the lochs (Figure 3.10). The $\delta^{13}C$ values of sediment trap samples are quite constant with time. The $\delta^{13}C$ values for transects of lochs Creran and Etive (Figure 3.10 b and c) show pronounced decrease from the head to the mouth of the lochs.

(a) Sediment trap.



(b) Loch Creran.



(c) Loch Etive.

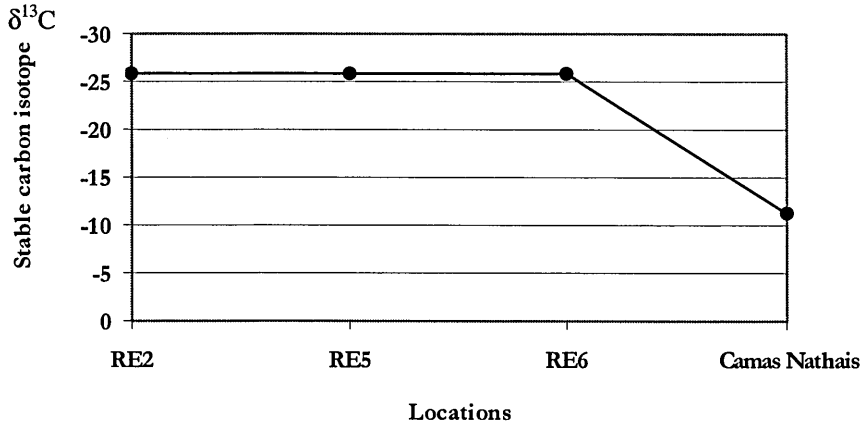


Figure 3.10 13C values for sediment trap, Loch Creran and Loch Etive.

3.5.3 Lignin and stable carbon isotopes

Regression analysis gives strong correlation between the $\delta^{13}C$ values and total lignin. Results for the regression analyses for the respective locations are as followed:

1. Sediment trap total lignin versus the $\delta^{13}C$ values: r= -0.68, r^2=0.47, $p<0.05$, n=13.
2. Transect-wise of Loch Creran, lignin versus $\delta^{13}C$ values: r= -0.76, r^2=0.58, $p<0.05$, n=109.
3. Transect-wise of Loch Etive, lignin versus $\delta^{13}C$ values: r= -0.96, r^2=0.93, $p<0.05$, n=4.

3.5.4 Summary

1. The sediment trap samples have a mean $\delta^{13}C$ value of –21.33‰.
2. In Loch Creran the $\delta^{13}C$ values ranged from –25.21‰ at LC0 to –14.30‰ at LC6.
3. In Loch Etive, the $\delta^{13}C$ values ranged from –25.70‰ at RE2, RE5 and RE6 to –11.25‰ at Camas Nathais.
4. The $\delta^{13}C$ values for sediment trap samples show some elevation during the summer.
5. For other locations, the $\delta^{13}C$ values are consistent throughout the year.
6. The $\delta^{13}C$ values increase significantly (single factor ANOVA: $p<0.05$) from the head to the mouth of both lochs.
7. The $\delta^{13}C$ values have significant (simple regression analysis: $p<0.05$) correlation with total lignin from the sediment trap (r=-0.68), and Loch Creran (r=-0.76) and Loch Etive (r=-0.96) transects sediments.

3.6 CN ANALYSIS

3.6.1 Total carbon and total organic carbon

Table 3.15a shows the H_2SO_4 treated and non-treated sediments subjected to CN analysis, table (b) shows the CN analysis of samples subjected to combustion at 500°C, and the table (c) shows the CN analysis of some untreated plant samples.

There is no significant difference between the %N in sediments treated and non-treated with H_2SO_4 (Table 3.15a), hence there is no nitrogen lost after the acid treatment. The carbon lost after the acid treatment is the inorganic carbon fraction. Only sediments from these locations: LC5, LC6, RE5 and Camas Nathais, have significant differences between the total carbon treated and non-treated with the acid. This shows that sediments from further down and outside the lochs have higher carbonate fraction.

After combustion at 500°C, most of the nitrogen contents of the sediments have gone (Table 3.16b). Similarly, Overnell and Young (1995) found that there was no carbon in the sample after heating at 450°C for 24h, hence all carbon in the sample was assumed to be organic. For LC0, LC1, LC2, LC5, RE2, RE5 and RE6 sediments, only slight percentages of carbon remained. Hence, the percentage weight loss after combustion at 500°C also consists of weight loss of the total carbon. Sediments from LC3, LC6 and Camas Nathais (CN; Table 3.15) have the highest %C following combustion at 500°C. These are the inorganic carbon fractions indicating that sediments further down the loch contain higher carbonate contents.

The %TC and %N, and C/N ratios for some plant samples were also obtained (Table 3.15c). These plant samples were not treated with H_2SO_4 so the total carbon includes the organic carbon. Bracken and birch stems have higher %TC but lower %TN than their leaves resulting in higher C/N ratios in the stem samples.

Table 3.15 C and N results. In (a), the ratio of the %TC/%TOC for each location is calculated. The %TC for all the future samples is then corrected to %TOC using these ratios.

(a) Percentages C and N, and C/N ratio for sediments treated and non-treated with H_2SO_4.

Location	Original (samples non-treated with H_2SO_4)			Samples treated with H_2SO_4			% reduction of %TC to %TOC	%TC/%TOC
	%TC	%TN	C/N	%TOC	%TN	C/N		
LC0	5.26	0.48	10.96	4.92	0.48	10.25	6.46	1.07
LC1	6.47	0.68	9.51	5.68	0.64	8.88	12.21	1.14
LC2	3.92	0.36	10.89	3.45	0.37	9.32	11.99	1.14
LC3	3.37	0.33	10.21	2.31	0.31	7.45	31.45	1.46
LC5	1.01	0.07	14.43	0.71	0.09	7.89	29.70	1.42
LC6	2.99	0.21	14.24	1.66	0.21	7.90	44.48	1.80
RE2	6.71	0.48	13.98	6.48	0.47	13.79	3.43	1.04
RE5	5.30	0.44	12.05	4.63	0.42	11.02	12.64	1.14
RE6	5.67	0.54	10.50	4.84	0.49	9.88	14.64	1.17
CN	3.49	0.28	12.46	2.11	0.27	7.81	39.54	2.11

The %TC obtained from the original sediments are the total carbon, whereas the total organic carbon (TOC) is obtained from sediment treated with H_2SO_4 to remove carbonates. Vales obtained here are mean values of a duplicate analysis.

(b) Percentages TC and N for sediments after combustion at 500°C.

Sample	% TC	% N
LC0	0.05	0.00
LC1	0.07	0.00
LC2	0.07	-0.01
LC3	0.10	-0.01
LC5	0.04	-0.01
LC6	0.14	0.00
RE2	0.05	-0.02
RE5	0.06	-0.02
RE6	0.07	0.00
CN	0.24	0.02

(c) Percentages C and N, and C/N ratio for plant samples.

Sample	%TC	%N	C/N
Bracken stems	22.81	0.36	63.36
Bracken leaves	21.99	2.16	10.18
Birch stems	25.05	0.75	33.40
Birch leaves	22.64	1.78	12.72
Spruce needles	23.40	0.94	24.89

3.6.2 Sediment depth profiles

Sediment from LC1 (12.12.2001) was analysed for C and N from 0-1cm layer increments down to 9-10cm layers (Table 3.16). The symbols ">" and "<" indicate that the parameter is significantly higher and lower than that in the following month. The %TC and %TN decreased and the C/N ratio increased gradually from the 0-1cm to the 9-10cm sediment layer.

Table 3.16 CN results for sediment depth profiles. This sample analysed was collected from LC1 on 12.12.2001. The %TOC and C/N corrected to %TOC/TN are given in parentheses. The %TOC values are obtained by dividing %TC with 1.14 (using the %TC/%TOC value for LC1 from Table 3.15a).

Depth (cm)	%TC			%TN			C/N
		Ave±probable uncertainty	% uncert		Ave±probable uncertainty	% uncert	
0-1	5.81	5.61 ±0.077	1.373	0.55	0.52 ±0.015	2.964	10.79
	5.45	(4.92)		0.48			(9.46)
	5.57			0.53			
1-2	5.87	5.87 ±0.104	1.772	0.54	0.57 ±0.019	3.411	10.30
	6.15	(5.15)		0.62			(9.04)
	5.61			0.54			
2-3	5.81	5.42 ±0.150	2.768	0.51	0.48 ±0.013	2.771	11.29
	5.12	(4.75)		0.45			(9.90)
	5.33			0.49			
3-4	4.74	4.89 ±0.067	1.370	0.35	0.36 ±0.004	1.083	13.58
	5.06	(4.29)		0.37			(11.92)
	4.86			0.36			
4-5	5.78	5.68 ±0.062	1.092	0.52	0.51 ±0.004	0.765	11.34
	5.74	(4.98)		0.50			(9.76)
	5.52			0.51			
5-6	4.77	4.71 ±0.052	1.104	0.41	0.37 ±0.016	4.216	12.73
	4.58	(4.13)		0.34			(11.16)
	4.79			0.36			
6-7	4.31	4.35 ±0.067	1.540	0.36	0.35 ±0.013	3.800	12.43
	4.22	(3.82)		0.38			(10.91)
	4.53			0.32			
7-8	4.19	4.12 ±0.023	0.558	0.30	0.30 ±0.002	0.633	13.73
	4.08	(3.61)		0.30			(12.03)
	4.11			0.29			
8-9	4.12	4.13 ±0.012	0.291	0.30	0.29 ±0.004	1.345	14.24
	4.16	(3.62)		0.28			(12.48)
	4.11			0.29			
9-10	4.56	4.15 ±0.273	6.586	0.31	0.31 ±0.004	1.258	13.39
	4.01	(3.64)		0.30			(11.74)
	3.88			0.32			

Ave = average; stdev = standard deviation; CV = coefficient of variation.

Hence in future only the 0-1cm and 9-10cm layers were subjected to analysis. Also in future, the samples are not subjected to acid treatment, as the total carbon contents of the sediments can be corrected to total organic carbon contents.

3.6.3 Sediment samples

The total carbon (TC) and total nitrogen (TN) were determined. Results are duplicate analyses of the same sample for the sediment trap samples. For other samples, it was triplicate analysis, consisting of three cores from the same locations. Results are given in Table 3.17, and the parameter which is significantly higher or lower than that of the following month is represented by the signs ">" and "<".

Table 3.17 %TC, %TN and C/N ratio.

(a) Sediment trap samples. The ">" and "<" signs indicate significantly higher or lower than the parameter in the following month.

Sample	%TC				%TN				C/N
	%TC	Mean ± probable uncertainty		% uncertainty	%TN	Mean ± probable uncertainty		% uncertainty	
Dec, 01	3.98	4.18	±0.14	3.35	0.41	0.41	±0.004	0.98	10.20
	4.38	<			0.42	<			
Jan, 02	5.20	5.20	0	0	0.56	0.56	0	0	9.29
	5.20	<			0.56	<			
Feb, 02	6.81	6.53	±0.20	3.06	0.66	0.65	±0.01	1.08	10.05
	6.25				0.64				
Mac, 02	6.00	6.54	±0.38	5.81	0.63	0.81	±0.13	16.05	8.07
	7.07				0.98				
Apr, 02	6.03	6.64	±0.44	6.63	0.85	0.93	±0.06	6.45	7.14
	7.26				1.01				
May, 02	7.98	7.90	±0.06	0.76	1.09	1.07	±0.02	1.98	7.38
	7.82				1.04				
June, 02	7.82	7.83	±0.01	0.13	1.04	1.03	±0.01	1.36	7.60
	7.84				1.01				
Aug, 02	6.81	7.16	±0.25	3.49	0.94	0.96	±0.01	1.46	7.46
	7.50				0.98				
Sep, 02	6.08	6.60	±0.37	5.61	0.80	0.81	±0.01	1.71	8.15
	7.12				0.83				
Oct, 02	7.65	6.85	±0.57	8.32	0.92	0.82	±0.07	8.66	8.35
	6.05				0.72				
Nov, 02	6.12	5.41	±0.50	9.24	0.72	0.63	±0.06	10.16	8.59
	4.70				0.54				
Dec, 02	5.36	5.77	±0.29	5.03	0.62	0.67	±0.04	5.97	8.61
	6.18				0.72				

(b) LC1. The %TOC and OC/N values are given in parentheses. The ">" and "<" are only calculated for the original %TC and %TN. The %TOC values are obtained by dividing %TC with 1.14.

Sample	%TC				%TN				C/N
		Mean ±probable uncertainty		% uncertainty		Mean ±probable uncertainty		% uncertainty	
16.7.01	3.60	3.58	±0.07	1.87	0.38	0.36	±0.01	3.33	9.94
	3.41	(31.4)			0.37				(8.72)
	3.74				0.33				
29.8.01	4.15	3.76	±0.34	9.17	0.44	0.37	±0.04	9.47	9.95
	2.86	(3.30)			0.29				(8.92)
	4.26	<			0.42				
12.12.01	4.87	5.52	±0.25	2.57	0.48	0.55	±0.03	4.55	10.04
	6.06	(4.84)			0.61				(8.80)
	5.64				0.55				
8.1.02	5.67	5.88	±0.08	1.41	0.56	0.59	±0.01	2.20	9.97
	6.07	(5.16)			0.61	>			(8.75)
	5.91	>			0.61				
6.2.02	0.44	0.44	±0.003	0.80	0.07	0.07	0	0	6.29
	0.43	(0.39) <			0.07	<			(5.57)
4.4.02	5.65	5.32	±0.13	2.38	0.61	0.54	±0.03	5.37	9.85
	5.32	(4.67)			0.55				(8.65)
	4.99				0.47				
7.5.02	5.78	5.65	±0.09	0.63	0.59	0.56	±0.02	3.21	10.09
	5.52	(4.96)			0.54				(8.86)
4.6.02	5.42	5.30	±0.09	1.66	0.59	0.56	±0.02	3.16	8.28
	5.17	(4.65)			0.54				(8.30)
2.7.02	5.43	5.67	±0.17	2.93	0.58	0.64	±0.01	2.33	9.33
	5.90	(4.97)			0.62				(7.77)

(c) LC0. The %TOC and OC/N values are given in parentheses. The ">" and "<" are only calculated for the original %TC and %TN. The %TOC values are obtained by dividing %TC with 1.07.

Sample	%TC				%TN				C/N
		Mean ±probable uncertainty		% uncertainty		Mean ±probable uncertainty		% uncertainty	
7.3.02	5.73	5.68	±0.02	0.30	0.57	0.59	±0.01	2.20	9.63
	5.66	(5.31) <			0.62	<			(9.00)
	5.66				0.57				
4.4.02	6.17	6.04	±0.10	1.57	0.65	0.65	±0.004	0.54	9.29
	5.90	(5.64) >			0.64	>			(8.68)
4.6.02	5.44	5.37	±0.09	1.69	0.49	0.51	±0.03	4.81	10.53
	5.54	(5.02) >			0.58	>			(9.84)
	5.14				0.48				
14.11.01:									
0-1cm	4.18	4.37	±0.13	3.07	0.42	0.45	±0.02	4	9.71
	4.56	(4.08)			0.47				(9.07)
9-10cm	4.32	4.05	±0.19	4.62	0.41	0.38	±0.02	4.74	10.66
	3.79	(3.79)			0.36				(9.95)

(c) LC2. The %TOC and OC/N values are given in parentheses. The ">" and "<" are only calculated for the original %TC and %TN. The %TOC values are obtained by dividing %TC with 1.14.

Sample	%TC				%TN				C/N
		Mean ±probable uncertainty		% uncertainty		Mean ±probable uncertainty		% uncertainty	
7.3.02	4.16	4.11	±0.02	0.56	0.45	0.43	±0.01	2.33	9.56
	4.18	(3.61)			0.41				(8.40
	3.99				0.42				
4.4.02	3.93	3.97	±0.08	0.04	0.41	0.40	±0.004	1	9.93
	4.08	(3.48) >			0.40	>			(8.70)
	3.89				0.39				
2.9.02:									
0-1cm	3.10	2.93	±0.12	4.10	0.35	0.32	±0.02	5.63	9.16
	2.76	(2.57)			0.30				(8.03)
9-10cm	3.28	3.32	±0.03	0.96	0.36	0.37	±0.004	0.95	8.97
	3.37	(2.91)			0.37				(7.86)

(d) LC3. The %TOC and OC/N values are given in parentheses. The ">" and "<" are only calculated for the original %TC and %TN. The %TOC values are obtained by dividing %TC with 1.46.

Sample	%TC				%TN				C/N
		Mean ±probable uncertainty		% uncertainty		Mean ±probable uncertainty		% uncertainty	
16.7.01	3.60	3.58	±0.07	1.90	0.38	0.36	±0.01	3.33	9.94
	3.41	(2.45)			0.37				(6.81)
	3.74				0.33				
21.3.02	4.20	3.54	±0.46	13.08	0.35	0.29	±0.03	13.45	12.21
	2.89	(2.42)			0.24				(8.34)
7.5.02	2.76	2.72	±0.03	1.18	0.24	0.22	±0.01	5	12.36
	2.67	(1.86)			0.21				(8.45)
30.9.01									
0-1cm	2.06	1.60	±0.33	20.56	0.30	0.24	±0.05	19.17	6.67
	1.13	(1.10)			0.17				(4.58)
9-10cm	2.05	1.90	±0.11	5.95	0.29	0.27	±0.02	6.67	7.04
	1.73	(1.30)			0.24				(4.81)

(e) LC5. The %TOC and OC/N values are given in parentheses. The ">" and "<" are only calculated for the original %TC and %TN. The %TOC values are obtained by dividing %TC with 1.42.

Sample	%TC				%TN				C/N
		Mean ±probable uncertainty		%uncertainty		Mean ±probable uncertainty		%uncertainty	
29.8.01	1.04	1.06	±0.04	3.77	0.10	0.11	±0.01	5.27	9.64
	1.16	(0.75) <			0.12	<			(6.82)
	0.97				0.10				
6.2.02	4.63	4.70	±0.02	0.49	0.47	0.47	±0.002	0.48	10.00
	4.73	(3.31) >			0.47	>			(7.04)
	4.72				0.48				
21.3.02	1.29	1.31	±0.02	1.37	0.15	0.15	0		8.73
	1.34	(0.92) <			0.15	<			(6.13)
2.7.02	6.85	5.99	±0.60	10.03	0.73	0.64	±0.06	9.38	9.36
	5.15	(4.22)			0.56				(6.59)

(f) LC6. The %TOC and OC/N values are given in parentheses. The ">" and "<" are only calculated for the original %TC and %TN. The %TOC values are obtained by dividing %TC with 1.80.

Sample	%TC			%TN			C/N
		Mean ±probable uncertainty	% uncertainty		Mean ±probable uncertainty	% uncertainty	
21.3.02	2.80	2.91 ±0.08	2.78	0.27	0.28 ±0.01	2.50	10.39
	3.03	(1.62)>		0.29	>		(5.79)
1.8.02	1.28	1.33 ±0.04	2.63	0.18	0.18 ±0.004	2.22	7.39
	1.38	(0.74)		0.19			(4.11)
30.9.02	1.46	1.53 ±0.05	3.46	0.21	0.27 ±0.01	4.78	5.67
	1.61	(0.85)		0.24			(3.15)

(f) %TC and %TC for Loch Etive. The %TOC and OC/N values are given in parentheses. The ">" and "<" are only calculated for the original %TC and %TN. The %TOC values are obtained by dividing %TC with 1.04 for RE2, 1.14 for RE5, 1.17 for RE6 and 1.65 for Camas Nathais.

Sample	%TC			%TN			C/N
		Mean ±probable uncertainty	% uncertainty		Mean ±probable uncertainty	% uncertainty	
RE2(14.2.01)	6.33	6.26 ±0.13	2.06	0.46	0.44 ±0.02	4.77	14.23
	5.92	(6.02)		0.39			(13.68)
	6.52			0.48			
RE5(17.1.01)	5.73	5.56 ±0.08	1.49	0.54	0.50 ±0.02	3.4	11.12
	5.34	(4.88)		0.46			(9.76)
	5.60			0.49			
RE6(17.1.01)	6.10	5.75 ±0.13	2.31	0.58	0.56 ±0.01	2.18	10.27
	5.56	(4.91)		0.55			(8.77)
	5.60			0.53			
RE6(14.2.01)	5.94	5.83 ±0.05	0.89	0.54	0.54 ±0.02	3.09	10.80
	5.86	(4.98)		0.59			(9.22)
	5.70			0.51			
RE6(20.3.01)	5.54	5.73 ±0.13	2.29	0.51	0.53 ±0.04	7.37	10.81
	5.91	(4.90)		0.63			(9.25)
Camas Nathais	3.50	3.49 ±0.01	0.20	0.29	0.28 ±0.01	3.93	12.46
(20.3.01)	3.48	(2.12)		0.26			(7.57)

For the sediment trap samples, the increase of %TC and %TN and decrease of the C/N ratio from May to August 2002, indicate the presence of the fresher phytoplankton in the summer. After that, both %TC and %TN decreased gradually from September to December 2002, but the C/N ratios increased. For the LC1 sediments, overall the %TC, %TN and C/N values are quite constant throughout the year, except for the decrease seen during February 2002. All these indicate a constant input of terrestrial organic matter into LC1. For all the other locations, overall, the %TC and %TN for all individual locations in Loch Creran decreased towards the end of the year, except for LC5 where both increased towards year-end. All these indicate the transport of terrestrial materials from River Creran along the loch with time. Hence at the beginning of a year, the total carbon contents are higher in sediments at the head of the loch, and towards the end of the year, this carbon was transported down the loch.

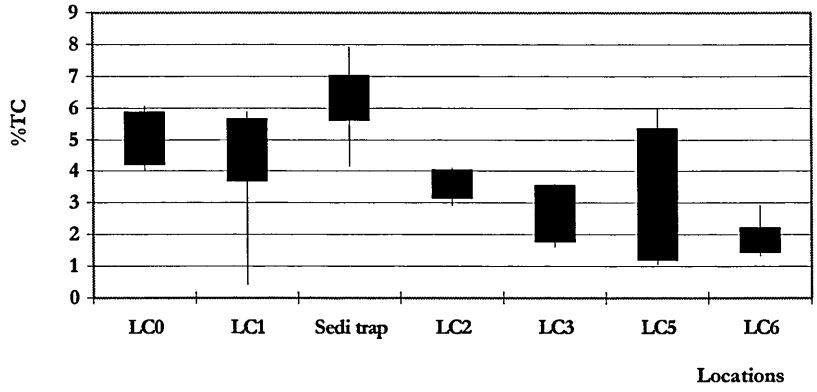
The %TC and %TN for Loch Etive were also determined (Table 3.17f). The locations RE2, RE5 and RE6 have significantly (single factor ANOVA: $p<0.05$) higher %TC and %TN compared to Camas Nathais. The terrestrial materials transported became more degraded, as shown in the highest C/N ratio of 12.46 at Camas Nathais. The mean values for %TC, %TN, %TOC, and OC/N for individual locations are given in Table 3.18. There are significant differences (single factor ANOVA: $p<0.05$) in the %TC and %TN across Loch Creran, but no significant difference ($p>0.05$) in the C/N ratios. In Loch Etive, there are significant differences in the %TC, %TN and C/N ratio along the loch.

Table 3.18 Mean values for %TC, %TN, %TOC, OC/N at individual locations.

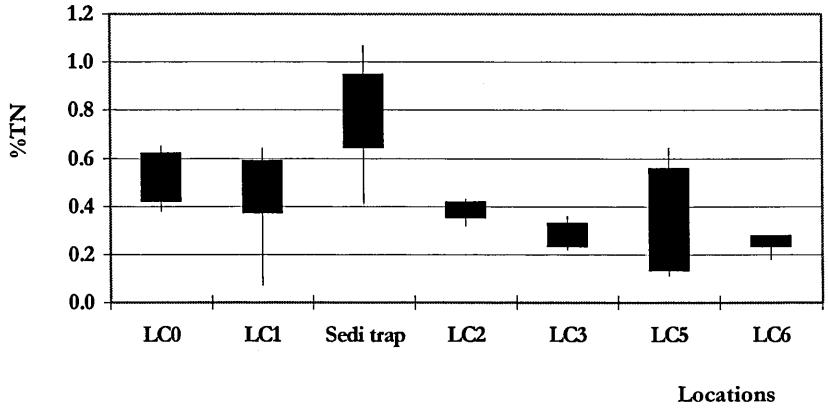
Location	%TC	%TN	C/N	%TOC	OC/N
Sediment trap	6.38	0.78	8.41		
LC0	5.10	0.52	9.96	4.77	9.31
LC1	4.57	0.47	9.30	4.01	8.26
LC2	3.58	0.38	9.41	3.14	8.25
LC3	2.67	0.28	9.64	1.83	6.60
LC5	3.27	0.34	9.43	2.30	6.65
LC6	1.92	0.24	7.82	1.07	4.32
RE2	6.26	0.44	14.23	6.02	13.68
RE5	5.56	0.50	11.12	4.88	9.76
RE6	5.77	0.54	10.63	4.93	9.08
Camas Nathais	3.49	0.28	12.46	2.12	7.57

Box plots of the %TC, %TN and C/N ratio of Loch Creran transect shows that the %TC and %TN are the highest in the sediment trap samples (Figure 3.11). This is most probably because the trap was deployed in the water column. Upon settling down some carbon has undergone degradation hence the lower %TC and %TN in the surface sediments from LC0 and LC1 and followed by LC5, LC2 and LC3. LC6 has the least abundances of %TC and %TN. From plot (c), it seems that the C/N ratios are more constant throughout the loch. Transect-wise of Loch Creran, there are significant differences (ANOVA, $p<0.05$) in the %TC and %TN across the loch. There is however, no significant difference ($p>0.05$) in the C/N ratios along the Loch Creran transect. In Loch Etive, there are significant differences ($p<0.05$) for the %TC, %TN and C/N ratios along the loch.

(a) %TC.



(b) %TN.



(c) C/N ratio.

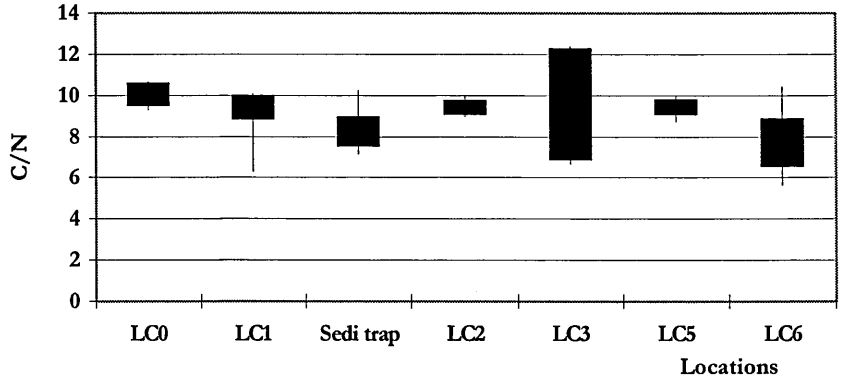


Figure 3.11 Box plots for %TC, %TN and C/N ratio for Loch Creran.

Overall there is mostly no significant correlation between total lignin with the %TC, %TN and C/N ratio. There are significant correlations ($p<0.05$) between total lignin with %TC at LC2, and Lochs Creran and Etive transects. Significant correlation between total lignin with %TN occurs only in Loch Creran transect. Also, significant correlation between total lignin and C/N ratio occur in sediment trap and LC3 sediment. Overall there is no significant correlation between the C/N ratio with total lignin and (Ad/Al)v as all these three parameters at individual location are quite constant with time.

There is strong correlation of total lignin with %TOC indicating that the terrestrial organic matter contributes to a considerable amount of TOC in Loch Creran. Both %TOC ($r=0.98$, $r^2=0.97$, $p<0.05$, $n=6$) and %TC ($r=0.98$, $r^2=0.95$, $p<0.05$, $n=7$) in Loch Creran has significant correlation with %TN, but not for Loch Etive.

A plot of % OC versus % TN for Loch Creran is shown in Figure 3.12. There is strong correlation ($r^2=0.97$) between %OC and %TN, indicating that most of the total nitrogen is organic. The slope of the correlation line (%OC/%TN = 9.71) corresponds to the average C/N ratio of 9.71.

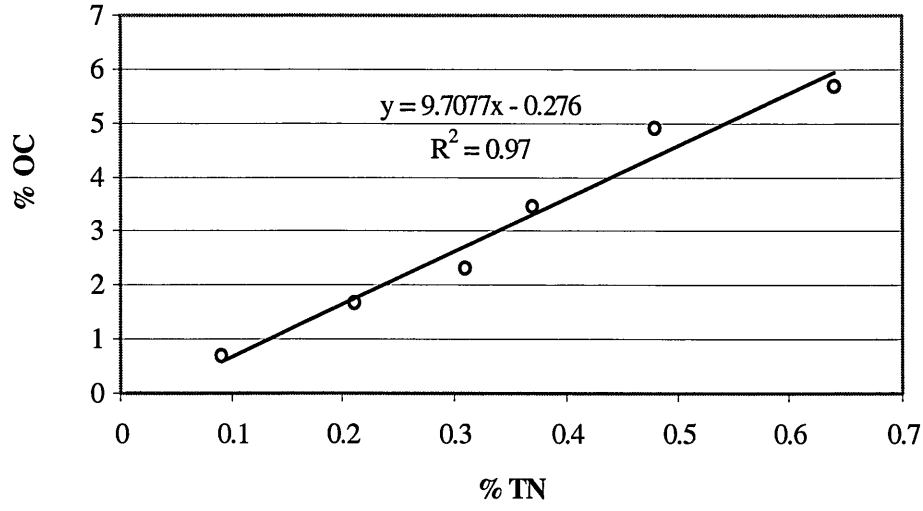


Figure 3.12 %OC versus %TN for transect of Loch Creran.

3.6.4 Lignin versus C, N and C/N

The results of the regression analyses between total lignin with %TC, %TN and C/N are given in Table 3.19a. Results of the regression analyses between the C/N ratio with total lignin and (Ad/Al)v are given in Table 3.19b.

Table 3.19 Regression analyses between lignin parameters and CN results.

(a) Between total lignin with both %TC and %TN.

Samples	Results for the regression analyses between total lignin and the %TC and %TN:	
	%TC	%TN
Sediment trap	r=-0.37, r^2=0.14, p>0.05, n=12	r=-0.54, r^2=0.29, p>0.05, n=12
LC1	r=0.74, r^2=0.55, p>0.05, n=3	r=0.69, r^2=0.48, p>0.05, n=3
LC0	r=0.94, r^2=0.88, p>0.05, n=4	r=0.93, r^2=0.86, p>0.05, n=4
LC2	r=0.99, r^2=0.99, p<0.05, n=3	r=0.97, r^2=0.94, p>0.05, n=3
LC3	r=-0.98, r^2=0.97, p>0.05, n=3	r=0.75, r^2=0.56, p>0.05, n=3
LC6	r=-0.79, r^2=0.63, p>0.05, n=3	r=-0.08, r^2=0.01, P>0.05, n=3
Loch Creran transect	r=0.94, r^2=0.88, p<0.05, n=7	r=0.87, r^2=0.76, p<0.05, n=7
Loch Etive transect	r=0.99, r^2=0.99, p<0.05, n=4	r=0.82, r^2=0.67, p>0.05, n=4
Loch Creran transect	%TOC: r=0.85, r^2=0.73, p<0.05, n=6	
Loch Etive trasect	%TOC: r=0.95, r^2=0.91, p<0.05, n=4	

(b) Between C/N ratio with both total lignin and (Ad/Al)v.

Samples	Results of the regression analyses between C/N ratio with the total lignin and (Ad/Al)v:	
	Total lignin (mg/g)	(Ad/Al)v
Sediment trap	r=0.63, r^2=0.39, p<0.05, n=12	r =-0.50, r^2=0.25, p>0.05, n=12
LC1	r =0.52, r^2=0.27, p>0.05, n=3	r =0.16, r^2=0.02, p>0.05, n=3
LC0	r =-0.51, r^2=0.24, p>0.05, n=4	r =-0.09, r^2=0.01, p>0.05, n=4
LC2	r =0.82, r^2=0.67, p>0.05, n=3	r =0.89, r^2=0.80, p>0.05, n=3
LC3	r =-0.99, r^2=0.99, p<0.05, n=3	r =-0.99, r^2=0.99, p>0.05, n=3
LC6	r =-0.99, r^2=0.97, p>0.05, n=3	r =0.65, r^2=0.43, p>0.05, n=3
Loch Creran transect	r =0.26, r^2=0.07, p>0.05, n=7	r =-0.31, r^2=0.09, p>0.05, n=3
Loch Etive transect	r =0.14, r^2=0.02, p>0.05, n=4	r =-0.19, r^2=0.04, p>0.05, n=4

3.6.5 Summary

1. In Loch Creran the %TC ranged 1.06-7.90%, %TN 0.11-1.07% and the C/N ratio 5.67-12.36%. In Loch Etive the %TC ranged 3.49-6.26%, %TN 0.28-0.56% and the C/N ratio 10.27-14.23.
2. The %TC, %TN and C/N ratio at individual locations are quite constant with time, and also with depth.
3. The %TC and %TN decreased significantly ($p<0.05$) from the head to the mouth of Loch Creran, while C/N ratios remained constant.
4. In Loch Etive, all %TC, %TN and the C/N ratio decreased significantly ($p<0.05$) from the head to the mouth of the loch.
5. Significant correlation occurred between total lignin with the %TC and %TOC in both lochs.

3.7 PHOSPHATE ANALYSIS

Results for the organic and inorganic phosphorus (P) contents are given in Table 3.20. The inorganic and organic P in the sediment trap samples increased during April, August and December 2002 (Table 3.20a). For LC1, the inorganic P increased during the later months, but there was no organic P present (Table 3.20b). There are no significant differences in the organic and inorganic P concentrations between the locations in Loch Creran.

Table 3.20 Results for organic and inorganic phosphate contents.

(a) Sediment trap samples.

Date	Inorganic P			Organic P		
	Concs (µg/g)	Mean ±probable uncertainty	% uncertainty	Concs (µg/g)	Mean ±probable uncertainty	% uncertainty
12.12.01	0.024 0.018	0.021 ±0.002	9.524	0.035 0.027	0.031±0.003	9.030
8.1.2001	0.09 0.116	0.103 ±0.009	8.740	0		
4.4.2002	0.68 0.622	0.651 ±0.021	3.231	0.067 0.085	0.076±0.006	3.421
1.8.2002	1.553 1.967	1.76 ±0.148	8.410	0.268 0.143	0.205±0.045	21.950
12.12.2002	0.688 0.656	0.672 ±0.011	1.620			

(b) LC1.

Date	Inorganic P				Organic P			
	Concs (µg/g)	Mean ±probable uncertainty		% uncertainty	Concs (µg/g)	Mean ±probable uncertainty		% uncertainty
6.2.2002	0.028 0.022	0.025	0.002	8.4	0.015 0.019	0.017	0.001	8.24
2.9.2002	0.018 0.018	0.018	0	0	0.002 0.004	0.003	0.001	33.33
30.9.2002	0.157 0.231	0.194	0.026	13.51				
12.12.2002	0.159 0.159	0.159	0	0				

(c) Other locations in Loch Creran.

Location	Date	Inorganic P				Organic P			
		Concs (µg/g)	Maen ± probable uncertainty		% uncertainty	Concs (µg/g)	Mean ±probable uncertainty		% uncertainty
LC2	2.9.2002	0.02 0.037	0.029	0.006	20.69	0.021 0.039	0.03	0.006	21.33
LC3	21.3.2002	0				0			
	30.9.2002	0.03 0.044	0.037	0.005	13.51	0.022 0.015	0.018	0.003	16.67
LC5	21.3.2002	0.011 0.011	0.011	0	0				
	12.12.2002	0.076 0.107	0.091	0.011	12.09	0.027 0.025	0.026	0.001	3.85
LC6	21.2.2002	0							
	30.9.2002	0.012 0.018	0.015	0.002	13.33	0.01 0.01	0.01	0	0

3.7.1 Lignin versus phosphate

Regression analysis gives significant ($p<0.05$) correlations between total lignin and inorganic phosphate ($r=-0.98$, $r^2=0.92$, $n=5$), and between total lignin and organic phosphate ($r=-0.98$, $r=0.97$, $n=5$) for the sediment trap samples.

3.8 OXYGEN UPTAKE RATE, LOI, Rp, C, N, C/N and P

Regression analyses between all the proxies to determine the biodegradability of sediment organic matter are carried out and results are given in Table 3.21. Table 3.21a gives a summary of the regression analysed between total lignin with all other proxies obtained from previous sections. Table 3.21b gives results for regression analyses between all the proxies. Only those with significant correlations (regression analysis: $p<0.05$) are given.

Table 3.21 Regression analyses for proxies for biodegradability of sediment organic matter. Only results for significant correlations are given. Otherwise, X (in parentheses) is used to denote that there is no significant correlation occurs.

(a) Regression analyses between lignin and other proxies.

Regression analysis between total lignin with these parameters:	Results for the regression analyses
$\delta^{13}C$	Sediment trap (r=-0.68), Loch Creran transect (r= -0.76), Loch Etive transect (r= -0.96)
Oxygen uptake rate	(X)
LOI	Loch Creran: % labile OM (r=0.98), %TOM (r=0.83), Rp (r= -0.95) Loch Etive: % labile OM (r=0.99), Rp (r= -0.99)
%TC	LC2, %TC (r=0.99) Loch Creran transect: %TC (r=0.94), %TN (r=0.87) Loch Etive transect: %TC (r=0.99) C/N: sediment trap (r=0.63) C/N: LC3 (r= -0.99)

(b) Regression analyses between all the proxies.

Regression analysis between:	With these parameters:
Oxygen uptake rate	% labile OM (X), % refractory OM (X), %TOM (X), Rp (X)
Oxygen uptake rate	%TC (X), %TN (X), C/N (X)
Oxygen uptake rate	$\delta^{13}C$ (X), IP (r=0.98, r^2=0.96, p<0.05, n=5), OP (X)
$\delta^{13}C$	% labile OM (X), % refractory OM (X), %TOM (X), Rp (r=0.86, r^2=0.74, p<0.05, n=7)
$\delta^{13}C$	%TC (X), %TN (X), C/N (X)
$\delta^{13}C$	IP (X), OP (X)
% labile OM	%TC (r=0.96, r^2=0.92, p<0.05, n=7), %TN (r=0.97, r^2=0.95, p<0.05, n=7), C/N (X)
% refractory OM	%TC (X), %TN (r=0.77, r^2=0.59, 0<0.05, n=7), C/N (X)
%TOM	%TC (r=0.91, r^2=0.82, p<0.05, n=7), %TN (r=0.91, r^2=0.82, p<0.05, n=7), C/N (X)
Rp	%TC (r=-0.95, r^2=0.91, p<0.05, n=7), %TN (r=-0.89, r^2=0.80, p<0.05, n=7), C/N (X)
IP	% labile OM (r=0.89, r^2=0.80, p<0.05, n=6), % refractory OM (X), %TOM (r=0.81, r^2=0.80, p<0.05, n=7), Rp (X)
OP	%labile OM (r=0.84, r^2=0.71, p<0.05, n=6), % refrac OM (X), %TOM (X), Rp (X)
IP	%TC (r=0.88, r^2=0.77, p<0.05, n=6), %TN (r=0.95, r^2=0.90, p<0.05, n=6), C/N (X)
OP	%TC (r=0.82, r^2=0.67, p<0.05, n=6), %TN (r=0.90, r^2=0.81, p<0.05, n=6), C/N (X)

3.8.1 Summary

1. Total lignin has significant correlation (simple regression analysis: $p<0.05$) with carbon isotope composition, % labile and % total organic matter, the Rp value, %TC and %TN and hence the C/N ratio.
2. The $\delta^{13}C$ values have significant correlation only with the Rp values.
3. The oxygen uptake rates do not have significant correlation with all other parameters, except with the inorganic phosphate.
4. As the total carbon and nitrogen are constituents of the percentage organic matter due to loss on ignition, there are strong correlation between the % labile and % total organic matter, and the Rp values with the %TC and %TN.

CHAPTER 4:DISCUSSION

4.1 GENERAL DISCUSSION

In this research, lignin was successfully elucidated in sediments from both Lochs Creran and Etive. Through detection of lignin materials in the sediments and by obtaining lateral distribution profiles for lignin in the lochs it was also demonstrated that lignin can be used as a biomarker for terrestrial organic matter in these systems. Based on the S/V and C/V ratios, the lignin oxidation products were also satisfactorily used to identify the vegetation sources as sedimenting material in the lochs. The quality, in terms of the degradation stage, of lignin material could also be determined by the parameters (Ad/Al)v, syringyl/vanillyl (S/V) and cinnamyl/vanillyl (C/V) ratios, as they can also indicate diagenesis. The lability of the bulk organic matter was investigated using some proxies used to determine the biodegradability of sediment organic matter. Combination of all these factors contributes to an understanding to the fate of the bulk organic matter in sea lochs. The hydrographic* and hydrodynamic+ regimes of the lochs, as well as the effect of bioturbation$^{\xi}$ on the sediment organic matter, control the overall quality and biogeochemical cycling of organic matter in the lochs.

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* **hydrographic** description of water properties, their distribution and variations.

+ **hydrodynamic** the effect of mechanical forces on a moving fluid.

$^{\xi}$ **bioturbation** a term derived from biological perturbation of soil and sediment and applied for activities of benthic organisms (macro-, meiobenthos) which result in reworked sediments (Baretta-Bekker *et al.*, 1998).

About this chapter

In this chapter, Section 4.2 focuses on the total lignin and (Ad/Al)v values in Loch Creran and Loch Etive in comparison with the total lignin and (Ad/Al)v values obtained from other locations worldwide. Reasons for the similarities and dissimilarities of the total lignin and (Ad/Al)v values between these two Scottish sea lochs with other locations from previous studies are provided. Lignin distribution and (Ad/Al)v values at individual locations and in lochs Creran and Etive transects are then studied. In Section 4.3, lignin oxidation products are used to determine the vegetation sources in both lochs Creran and Etive. These are then compared with aerial photographs of Loch Creran for further confirmation of the vegetation sources surrounding the loch. Finally the lignin phenols of some plant samples commonly found in the catchment of Loch Creran were investigated in order to confirm that certain tissue types yield certain lignin-derived phenols. In Section 4.4 the importance of the effect of terrestrial organic matter on the biodegradability of the sediment organic matter is discussed in relation with the proxies to determine the biodegradability of sediment organic matter. These proxies are the oxygen uptake rate, percentage organic matter due to loss on ignition, the Rp index, TC, TN, the C/N ratio, and P content. Section 4.5 investigates the contribution of lignin and terrestrial organic matter to total carbon, total organic carbon and total organic matter. The transport route of organic matter from River Creran along Loch Creran is magnified into details such as the processes occurring in the water column and sediment, and discussed in relation with the system and processes in the lochs (Section 4.6). Finally, using lignin as the biomarker, these combined parameters are employed to address the question of the fate of the terrestrial organic matter during their transport along the lochs. The effect of fish farms on the sediment organic matter content and its biodegradability is ruled out as the sampling sites lie beyond the influence of fish farm inputs. However, bioturbation may play an important role in governing the fate of bulk organic matter or terrestrial organic matter, and even the fate of the lignin material in the surface 0 to 10 cm sediment layer, especially in Loch Creran. Section 4.7 details the determination of carbon budgets within Loch Creran; the inputs and outputs of carbon and organic matter in the loch are determined, and this will be discussed in relation with the global carbon cycle.

4.2 LIGNIN DISTRIBUTION

4.2.1 Lignin and (Ad/Al)v around the world

4.2.1.1 Λ and total lignin (mg/g)

Hedges and Mann (1979b) first used Λ (Greek capital letter lambda) to indicate the sum of the syringyl, vanillyl and cinnamyl per 100mg OC [S+V+C (mg/100mg OC)]. Since then Λ has been used to define total lignin from various locations worldwide (Section 1.5.1.2). Total lignin in both Loch Creran and Loch Etive are firstly compared with total lignin in other locations worldwide. The parameter Λ is used to compare past and present results as this is most commonly used to define total lignin. In this research, total lignin was calculated as the sum of S+V+C in terms of mg/g dry weight of sediment sample (Sections 2.2.3.9 and 3.2). Since most of the past results were reported as Λ, data have now been normalized to mg/100mg OC (Λ). These Λ values are only estimations, as they are based on the mean %TOC calculated in Section 3.6 (the mean values for %TOC in Table 3.18 are calculated from results in Table 3.17) for individual locations. An example of the normalization of mg/g of total lignin to Λ (mg/100mgOC) of total lignin is shown here:

The %TOC = (mgOC in sediment sample/mg sediment) x 100%

For LC0, %TOC = 4.77%,

indicating that the OC content at LC0 = 4.77mg OC/100mg sediment

Hence in 1g of sediment sample, mg OC in the sample = 47.7mg

Hence if total lignin = 0.3305mg/g sediment, this is equivalent to 0.3305mg/47.7 mg OC

Normalizing to 100mg OC,

Lignin content = (0.3305mg x 100mg OC)/47.7mg OC = 0.6929mg per 100 mgOC

Hence Λ = 0.6929 (mg/100 mgOC)

The mean total lignin (mg/g) and %TOC for individual locations in both lochs Creran and Etive were used to calculate the Λ values for these locations. Table 4.1 shows the %TOC, mean total lignin (mg/g), and Λ (mg/100mg OC) for all the individual sampling locations.

Table 4.1 Total lignin (in mg/g and mg/100mg OC) for all sampling locations. The Λ is calculated from the %TOC and total lignin (mg/g). Refer text for calculation.

Locations	%TOC	Total lignin (mg/g)	Λ (mg/100mg OC)
LC0	4.77	0.3305	0.6929
LC1	4.01	0.2180	0.5436
LC2	3.14	0.1760	0.5605
LC3	1.83	0.0682	0.3727
LC5	2.30	0.1016	0.4417
LC6	1.07	0.0470	0.4393
RE2	6.02	0.5477	0.9098
RE5	4.88	0.4509	0.9240
RE6	4.93	0.4823	0.9783
Camas Nathais	2.13	0.0718	0.3387

Overall the Λ values in both lochs Creran and Etive ranged from 0.37 to 0.98. The Λ values from LC6 and Camas Nathais are not included in this range, although values for LC6 were within the Creran range, as both are situated outside the lochs and hence might be influenced by materials transporting outside the vicinities of these two lochs. These Λ values are then compared with the Λ values obtained from previous studies. The lignin parameters Λ, S/V, C/V and (Ad/Al)v, as well as other parameters such as %TOC, C/N and $\delta^{13}C$ values from sediments obtained from other locations from past studies are given in Table 4.2. The table is divided into three sections: (a) marine sediments (b) river to coastal/marine sediments (c) estuaries and other locations such as lake (and loch in this study). Comparison with previous studies shows that the Λ values in Loch Creran and Loch Etive are also within the Λ values reported in the past in the marine, riverine and estuarine sediments. However the (Ad/Al)v values in Loch Creran and Loch Etive are higher compared to other locations. Figure 4.1 shows the Λ and (Ad/Al)v values for sediment samples obtained from all the locations worldwide from the marine, coastal, riverine and estuarine sediments.

Table 4.2 Lignin and other parameters from past and present studies. All the parameters are given in mean, or ranges as reported by the respective authors. The S/V and (Ad/Al)v values in brackets are ranges when the values of the sediment trap samples are included. For other parameters, the values for the sediment traps samples fall within these ranges. The reason for this difference is due to the very high (Ad/Al)v values for the sediment trap samples (Section 4.2.1.3).

(a) Marine sediments.

No	References and locations.	Λ	S/V	C/V	(Ad/Al)v	%TOC	C/N	$\delta^{13}C$ (‰)
1	Hedges and Mann (1979b): Washington continental shelf and slope							
	- nearest mouth of the Columbia River	6.8±1.7	0.32±0.06	0.07±0.02		1.14±0.23		
	- furthest from the Columbia River	0.23±0.04	0.32±0.06	0.38±0.12		2.20±0.47		
2	Hedges and can Green (1982): Gulf of Mexico - Holocene	0.06						
	- Pleistocene	0.10						
	Washington slope – Holocene	0.24						
	- Pleistocene	0.39						
3	Prahl *et al.* (1994): Washington margin – continental shelf	3.52	0.26	0.05	0.28		14.5	-23.9
	- continental slope	0.75	0.30	0.12	0.33		10.5	-21.8
	- Cascadia Bay	0.26	0.46	0.22	0.41		9.2	-21.4
	- Columbia River basin	3.18	0.40	0.15	0.29		14.8	-25.7
	- Cascadia sea channel	0.92	0.37	0.11	0.40		11.2	-23.2
4	Goni *et al.* (1997): Gulf of Mexico	1.5	1.25-3.09	0.28-0.83	0.46-1.18			-21.7 to –19.7
5	Goni *et al.* (1998): Gulf of Mexico – transect A	0.81	1.51	0.46	0.61		10.75	-19.7
	- transect B	0.53	1.78	0.59	0.97		11.00	-21.7
6	Miltner and Emeis (2001): Baltic Sea	0.37	0.42	0.31	0.43			
7	Kastner and Goni (2003): Amazon basin late Pleistocene sediment	3.1	0.84	0.08				-27.6

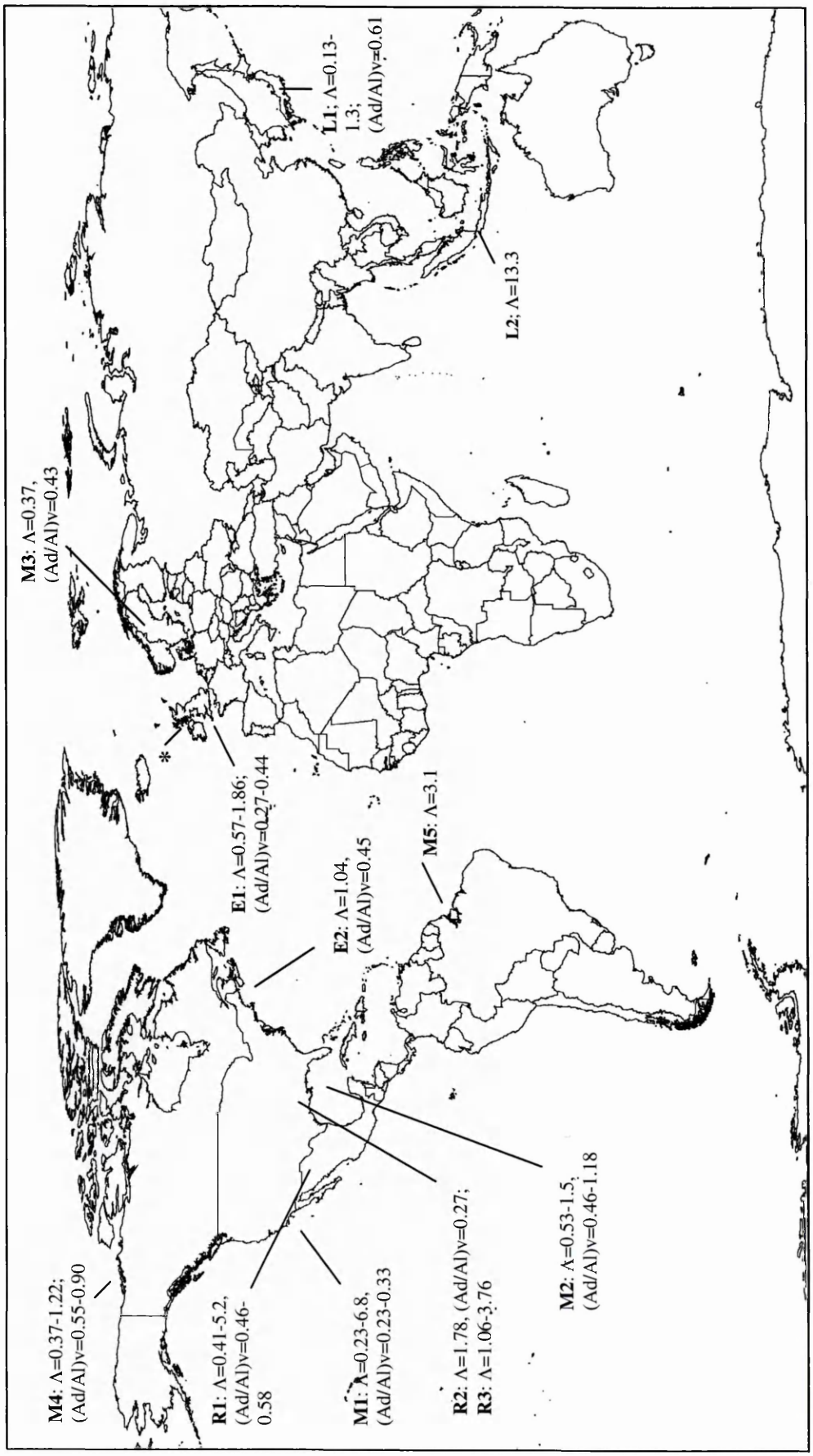
(b) River to coastal/marine sediments.

No	References and locations.	Λ	S/V	C/V	(Ad/Al)v	%TOC	C/N	$\delta^{13}C$ (‰)
1	Hedges *et al.* (1984): Columbia River	0.41-5.2 Mean=1.17	0.14-0.94 0.68	0.04-0.83		1.41	16.5±4.8	-24.8±1.2
2	Goni *et al.* (2000): - Beaufort shelf surface sediments	0.37-1.22	0.54-1.71	0.15-0.28	0.55-0.90			
3	Bianchi *et al.* (2002): - lower Mississippi River	0.71-3.74 Mean=1.78	0.93, 1.05	0.03, 0.10	0.27, 0.17		12.0±1.4	-21.9 to – 24.64 -23.20
	- Louisiana shelf sediments	0.68-1.36 Mean=0.54	0.84, 0.69	0.06, 0.05	0.31, 0.33		10.8, 9.9	-22.5 to – 21.2
4	Gordon and Goni (2003): - Atchafalaya delta and bay region - offshore	3.76 1.06					11.3 8.9	-23.5 -21.5

(c) Estuaries, lakes and loch.

No	References and locations.	Λ	S/V	C/V	(Ad/Al)v	%TOC	C/N	$\delta^{13}C$ (‰)
1	Readman *et al.* (1986): Tamar Estuary	0.57-1.86	0.25-0.40	0.07-0.25	0.27-0.44			
2	Requejo *et al.* (1986): Narragansett Bay Estuary	1.04	0.51	0.15	0.45			-22.54
	Lakes:							
1	Hedges *et al.* (1982): Lake Washington Late Quaternary sediment							
	Sample 0.06	1.66	0.44	0.22	0.58	4.25	11-14	
	Sample 7.4	1.78	0.80	0.34	0.46	5.43		
2	Ishiwatari and Uzaki (1987): Lake Biwa	0.13-1.3	0.53	0.14	0.61			-25 to –29
3	Tareq *et al.* (2004): Rawa Dawau Lake	13.3				3-56	16-39	-26.98 to –30.07
	Loch:							
	This study: Loch Creran and Loch Etive	0.37-0.98	0.15-1.00 (0.15-1.61)	0-1.47	0-1.58 (0-7.61)	1.83-6.02	6.60-13.68	-25.71 to –16.10

Chapter 4: DISCUSSION


M4: Λ=0.37-1.22; (Ad/Al)v=0.55-0.90
R1: Λ=0.41-5.2, (Ad/Al)v=0.46-0.58
M1: Λ=0.23-6.8, (Ad/Al)v=0.23-0.33
R2: Λ=1.78, (Ad/Al)v=0.27; **R3**: Λ=1.06-3.76
M2: Λ=0.53-1.5; (Ad/Al)v=0.46-1.18
M5: Λ=3.1
E2: Λ=1.04, (Ad/Al)v=0.45
E1: Λ=0.57-1.86; (Ad/Al)v=0.27-0.44
M3: Λ=0.37, (Ad/Al)v=0.43
L2: Λ=13.3
L1: Λ=0.13-1.3; (Ad/Al)v=0.61

Keys:

M1: Washington continental shelf and slope (Hedges and Mann, 1979b; Hedges and van Green, 1982; Prahl *et al.*, 1994)

M2: Gulf of Mexico (Goni *et al.*, 1997, 1998)

M3: Baltic Sea (Miltner and Emeis, 2001)

M4: Beaufort shelf (Goni *et al.*, 2000)

M5: Amazon basin late Pleistocene sediments (Kastner and Goni, 2003)

R1: Lake Washington and Columbia River (Hedges *et al.*, 1982; Hedges *et al.*, 1984)

R2: Lower Mississippi River (Bianchi *et al.*, 2002)

R3: Atchafalaya delta and bay region (Gordon and Goni, 2003)

E1: Tamar Estuary (Readman *et al.*, 1986)

E2: Narragansett Bay Estuary (Requejo *et al.*, 1986)

L1: Lake Biwa, Japan (Ishiwatari and Uzaki, 1987)

L2: Rawa Dawau Lake, Indonesia (Tareq *et al.*, 2004)

*: Our sampling locations, Loch Creran and Loch Etive

Λ is V+S+C (mg/100mg TOC); M=marine, R=river, E=estuary and L=lake sediments.

Figure 4.1 Worldwide Λ and (Ad/Al)v values. The references used for this map are compiled from results in Table 4.2. The map was obtained from Steve Gontarek (Dunstaffnage Marine Laboratory) from the Ordnance Survey, viewed at the Digimap service run by Edina (Crown Copyright/Digimap right 2005. All Ordnance Survey/Edina supplied service).

4.2.1.2 Λ values

The Λ values in Loch Creran and Loch Etive range from 0.37 to 0.98, within the Λ values reported in previous studies (0.06 to 6.8; see Table 4.2 and below). Some of the reasons why Λ was found to be consistent within a range in sediments obtained from throughout the world are discussed.

Implications

1. The highest Λ values were found nearest the mouth of the Columbia River (6.8 and 5.2; Hedges and Mann, 1979b; Hedges *et al.* 1984), followed by the Atchafalaya bay and delta regions (3.76; Gordon and Goni, 2003), the Washington margin continental shelf and the Columbia River basin (3.52 and 3.18, respectively; Prahl *et al.*, 1994) and the Amazon basin for the late Pleistocene sediments (3.1; Kastner and Goni, 2003). All these values are higher than the Λ values found in lochs Creran and Etive, as the highest Λ value in these two lochs is 0.98.

   The most probable reason for a high Λ value in the mouth of Columbia River would be due to accumulation of terrestrial materials in locations nearest the river mouth as will be discussed in Section 4.2.2.2. Another reason could be these locations are fuelled by rivers draining larger catchment areas than the River Creran and River Etive. For example, the Amazon River is the world largest river (Hedges *et al.*, 1986) draining the lowland tropical forests into the Amazon basin (Richey *et al.*, 2002). Loch Creran and Loch Etive both drain smaller catchment areas than these locations.

2. The Λ values from these locations are slightly higher than lochs Creran and Etive: Gulf of Mexico, Beaufort shelf sediments, Louisiana shelf sediments, offshore from the Atchafalaya bay, Tamar Estuary, Narragansett Bay Estuary and Lake Washington

sediments (see Table 4.2). High rainfall plus these locations drain larger catchment area than lochs Creran and Etive could be the important reasons.

3. The Λ values from lochs Creran and Etive are however, mostly higher than the Λ values obtained from some marine environments such as: a location furthest from the Columbia River in the Washington shelf (0.23; Hedges and Mann, 1979b), the Washington slope Holocene and Pleistocene sediments (0.24 and 0.39, respectively; Hedges and van Green, 1982), the Washington continental slope, Cascadia Bay and Cascadia sea channel (0.75, 0.26 and 0.92; Prahl *et al.*, 1994), the Gulf of Mexico (0.53 to 1.5; Goni *et al.*, 1997 and 1998), and the Baltic Sea (0.37; Miltner and Emeis, 2001). The lowest Λ value in the marine environments was found in the Gulf of Mexico Holocene and Pleistocene sediments (Λ = 0.06 and 0.10, respectively; Hedges and van Green, 1982).

   The most probable explanation will be that these marine locations are situated further from the river source; hence the lignin materials could have been deposited into the sediments before being transported to these locations. Further towards the sea, the lignin materials in these sediments were diluted with marine organic matter.

4. Most of the systems studied: marine, coastal and estuaries are fuelled by riverine input of terrestrial materials. During the journey these materials could have been lost through degradation, accumulation and sedimentation. Hence in all these locations, as well as in lochs Creran and Etive, the Λ values do not exceed the highest reported Λ values of 6.8. The Λ values from the Rawa Dawau Lake is exceptional (13.3), hence not included in this range.

   Besides, most of the areas studied are open water systems, where the terrestrial materials are transported seaward until only a trace amount is detected. Similar circumstances also apply for the lignin material. The reason these materials do not accumulate to exceeding the range 0.06 to 6.8, is due to a continuous transport of materials further towards the open sea.

Even though they might be accumulated in one location, some of the lignin materials accumulated would have undergone diagenesis to form humus (Hurst and Burges, 1967; Christman and Oglesby, 1971; Zeikus, 1980; Hedges and Oades, 1997), hence will not always exist as lignin.

5. Of the three lakes studied: Lake Washington, Lake Biwa and Rawa Dawau Lake, the reason that the Λ value (mean=13.3) was much higher in Rawa Dawau Lake could be due to the input from the more dense and greater variety of vegetation from the tropical swamp forests surrounding this lake compared to Lake Washington and Lake Biwa (Hedges *et al.*, 1982; Ishiwatari and Uzaki, 1987; Tareq *et al.*, 2004).

6. As lakes are low energy hydrographic regimes and closed systems, with limited outflow, most of the lignin materials could then be retained in the Rawa Dawau Lake, hence the high Λ value of 13.3. As both lochs Creran and Etive, as well as all other locations are open systems, the lignin material will be transported to the sea, hence the lower Λ compared to Rawa Dawau Lake.

4.2.1.3 (Ad/Al)v values

The (Ad/Al)v ratios reflect the degree of microbial alteration of lignins as a high (Ad/Al)v value indicates extensive degradation of the lignin components in aerobic terrestrial environments (Ertel and Hedges, 1984; Hedges and Weliky, 1989). The elevated (Ad/Al)v value of more than 0.5 indicates highly degraded materials (Goni *et al.*, 2000). The (Ad/Al)v values in both lochs Creran and Etive range from 0 to 7.61, these values seem to be higher in these two lochs compared to other locations (Table 4.2).

It is found that the high maximum value of the (Ad/Al)v is due to the increase of (Ad/Al)v value in the sediment trap in July 2002 [(Ad/Al)v=7.61; Table 3.2a]. Omitting these values, the range

of (Ad/Al)v values for Loch Creran is 0-1.58, and for Loch Etive 0.52-1.23. For Loch Creran, (Ad/Al)v=0 occurred at LC5 when C=0 during February 2002 (Table 3.2f). Omitting the "0" value, the (Ad/Al)v values in Loch Creran range 0.32-1.58. Omitting the 7.61 value, the (Ad/Al)v in sediment trap samples ranged from 0.76-4.75. Hence even when omitting the highest (Ad/Al)v value of 7.61, the (Ad/Al)v values in these two lochs are still slightly higher than most (Ad/Al)v values from other locations (Table 4.2). Some explanations are offered below.

Implications

1. One explanation could be because of low sedimentation/deposition rate, as this gives more time for decomposition to occur, as the more labile substances are utilized more rapidly (Canfield, 1994). But because for some sediment cores, the bottom 9-10cm sediment layers sometimes seemed to contain less degraded materials [as indicated by the lower (Ad/Al)v value] than the surface 0-1cm sediment (see Table 4.19), this reason is ruled out. In fact, the presence of the 'more recent or fresher' lignin materials in the bottom sediments indicates very high sedimentation rate occurring in Loch Creran.

   The mean sedimentation rate calculated from the sediment trap samples is 11.11 $g/m^2/day$ (Sections 2.2.1.2 and 3.1.2). As the mean %TC of the sediment trap sample is 6.38%, the sedimentation rate is also equivalent to 240.9 $gC/m^2/year$. The calculation is as shown below.

   Sedimentation rate = 11.11 $g/m^2/d$

   Mean %TC for sediment trap (Table 3.18) = 6.38%

   As %TOC is usually less than %TC (Tables 3.15a and 3.18), %TOC for the sediment trap samples is estimated from %TC by dividing a factor of 1.07 (as %TC/%TOC = 1.07 for LC0 sediment sample, and LC0 was situated nearest the sediment trap).

So, mean %TOC for sediment trap samples = 6.38%/1.07 = 5.96%

Hence, in 100g sediment there is 5.96g OC

In 11.11g sediment, there is (5.96g OC/100g) x 11.11g = 0.66g OC

So, sedimentation rate = 11.11 g/m^2/d ≡ 0.66 gOC/m^2/d = 240.9 gOC/m^2/year

Comparison of this mean sedimentation rate in the uppermost basin of Loch Creran with the sedimentation rates obtained from other locations (Table 4.3) shows that the sedimentation rate in this loch is higher than other locations worldwide. According to Henrichs (1992), a high sedimentation rate is one above 0.1cm/yr and also more than 100g C/m^2/d. According to Howe (*pers. comm.*) the average sedimentation rate in Loch Creran is 0.6 cm/yr. From this work, the mean sedimentation rate in Loch Creran was found to be 240.9g OC/m^2/year. Both indicate high sedimentation rate in Loch Creran. Requejo *et al.* (1986) found that the sedimentation rate in Narragansett Bay Estuary ranged from 1.4-2.0 cm/yr.

Table 4.3 Sedimentation rates obtained from past studies.

References (year)	Location	Sedimentation rate
Ansell (1974)	RE6 (equivalent)	0.008-0.684 gC/m^2/d
Rowe and Gardner (1979)	Northwest Atlantic Ocean	0.0128 gC/m^2/d
Rowe *et al.* (1986)	Continental shelf eastern US south of New England	0.128 gC/m^2/d
Cronin and Tyler (1980)	Loch Creran	50 gC/m^2/y
Vangriesheim and Khripounoff (1990)	Meriadzek Terrace, Bay of Biscay	1.6 gC/m^2/y
De Haas and Van Weering (1997)	Entire Skagerrak/northern Kattegat	35 gC/m^2/y
This study	Loch Creran	0.66 gC/m^2/d = 240.9 gC/m^2/yr

Sedimentation rates were calculated in gC/m^2/d and gC/m^2/yr. The sedimentation rate in Loch Creran is reported in both.

Comparison with the sedimentation rate obtained by Cronin and Tyler (1980) indicates that the mean sedimentation rate in the uppermost basin of Loch Creran of 240.9 $gC/m^2/yr$ is quite reasonable. Cronin and Tyler (1980) found that the annual sedimentation rate in the main basin in the middle of Loch Creran was 50 $gC/m^2/yr$. This was about five times lower than the sedimentation rate obtained in this study, the reason being our sediment trap was deployed nearer to the river source in the upper most basin of the loch, hence receiving greater input of terrestrial debris from River Creran.

The high sedimentation rate in Loch Creran results in high burial efficiencies hence the fresher materials trapped in the bottom 9-10cm sediment layers. Sediment accumulation rate affects the reactivity and ultimate preservation of organic matter as fast deposition more rapidly moves organic matter down through this diagenetically active zone with less total degradation (Hedges and Keil, 1995).

The reason for the high sedimentation rate in the uppermost basin of Loch Creran is because Loch Creran is a small loch with rapid exchange and mixing with the outside seawater, and good tidal flushing to ensure ventilation of the bottom water (Section 1.3.2; Gage, 1972).

2. The continuous water circulation resulting in oxic conditions in the water column of the loch, enabling these terrestrial materials to be degraded continuously. Neither Loch Creran nor Loch Etive upper basins go anaerobic (this work, Edwards and Truesdale, 1997 and Overnell *et al.*, 2002) and this was also the situation 30 years ago (Gage, 1972).

3. The (Ad/Al)v value did not alter much for the lignin material whilst in the lochs, this means that the lignin material has already been degraded before entering the lochs. Besides, the acid/aldehyde ratio does not increase much in the aquatic environments (Hedges *et al.*, 1986; Ertel *et al.*, 1986; Hamilton and Hedges, 1988: Section 1.4.4).

4. Another reason could be due to the vegetation type in these two lochs. The vegetation in both lochs Creran and Etive consists of deciduous plants (non-woody angiosperm tissues). Previous authors (Holt and Jones, 1983; Hedges *et al.*, 1985; Hedges *et al.*, 1988a) had reported that angiosperm tissues are more susceptible to degradation compared to gymnosperm tissues. This is most probably because the syringyl and cinnamyl phenols are preferentially degraded compared to the vanillyl phenols (Hedges *et al.*, 1985; Ertel *et al.*, 1986; Hedges *et al.*, 1986; Hedges *et al.*, 1988c; Lobbes *et al.*, 2000; Dittmar and Lara, 2001), as non-woody angiosperm produces syringyl, vanillyl and cinnamyl phenols and the gymnosperm tissues produce vanillyl phenols. Also, according to Nelson *et al.* (1995), soft-rot fungi degrade gymnosperm to a lesser extent than angiosperm lignin (Section 1.4.3.3).

   Hence this leads to the question of whether the (Ad/Al)v values for angiosperm tissues are affected; as the (Ad/Al)v is the ratio of the vanillic acid to vanillin, and both are from the vanillyl phenol group.

   In some locations the vegetation sources consist of gymnosperm tissues (Hedges and Mann, 1979b; Hedges and van Green, 1982; Hedges *et al.*, 1982; Requejo *et al.*, 1986; Ishiwatari and Uzaki, 1987). The decomposition of the gymnosperm was slower, and the (Ad/Al)v values reported were not as elevated as the (Ad/Al)v values reported in this study. In fact, only in lochs Creran and Etive, the Gulf of Mexico and North West Mediterranean and North East Atlantic (Gough *et al.*, 1993; Section 2.2.3.10, Implication 4), the (Ad/Al)v values exceed "1" (Table 4.2), and in these three locations the non-woody angiosperms predominate (this study; Gough *et al.*, 1993; Goni *et al.*, 1997).

   This could mean that on the whole even the vanillyl phenols of the angiosperm tissues are more degradable than the gymnosperm tissues vanillyl phenol. There is however no published literature to support this. Alternatively, the slightly higher (Ad/Al)v values in lochs Creran and Etive could be explained as in reasons 2 and 3.

5. The extremely high (Ad/Al)v values found in the sediment trap samples can be explained following Reeves and Preston (1991), whereby permanently suspended particles contain more lignin fraction than the surface sediments (see Section 4.6.1.2). This phenomenon would be particularly expected in Loch Creran where the outflowing freshwater is entrained by the inflowing saline water, creating a permanently suspended layer.

6. The (Ad/Al)v values were higher compared to values from previous studies, yet the $\delta^{13}C$ values did not seem to be too depleted in sediments from lochs Creran and Etive compared to $\delta^{13}C$ values from past studies.

   The (Ad/Al)v ratio is due to the ratio of vanillic acid to vanillin, both of them are lignin components, hence the higher (Ad/Al)v values indicate greater diagenesis of the lignin material and that the lignin material in these two lochs is highly degraded.

   The carbon isotope composition represents the measurements for the bulk organic matter, not the lignin material. Besides, the $\delta^{13}C$ values do not change during decomposition of the phytoplankton (Gearing *et al.*, 1984). Hence the $\delta^{13}C$ values are measurements for the relative abundances between terrestrial and marine organic matter.

7. The effect of superoxidation on the (Ad/Al)v values is explained in Section 2.2.3.10.

4.2.1.4 Other parameters

All the lignin parameters and some other parameters commonly used to define terrestrial organic matter such as %TOC, C/N ratio and $\delta^{13}C$ values from the past are tabulated (Table 4.2). The %TOC, C/N and $\delta^{13}C$ values in these two lochs are all within the ranges reported in sediments from past studies (Table 4.2).

4.2.2 Lignin and (Ad/Al)v in Loch Creran and Loch Etive

4.2.2.1 Constancy at individual locations

There was continuous presence of lignin materials in Loch Creran. Table 3.2a, b, c, d, e and g show the presence of all individual lignin-derived phenols in the sediment trap, LC1, LC0, LC2, LC3 and LC6, except for LC5 (Table 3.2.f) where there were several lignin phenols not detected during February 2002. Only locations in Loch Creran are discussed here as this loch was sampled for one year, whereas Loch Etive was sampled for only three months. Results from Loch Etive can be compared and discussed in relation to results from Loch Creran. At individual locations in Loch Creran, there were mostly no significant differences (ANOVA: $p>0.05$) in all the lignin parameters between subsequent sampling months (Table 3.2; Section 3.2).

Sediments from several other geographic regions were also found to contain stable lignin materials, some of them for several hundred to million years: Hedges and Mann (1979b), Hedges *et al.* (1982, 1985), Ishiwatari and Uzaki (1987) and Kastner and Goni (2003). All these findings (Table 4.4) indicate the stability of the lignin components and minimal *in situ* lignin degradation, especially in the aquatic environments (Sections 1.4.3-1.4.5) and as would be expected in anaerobic sediments, although ultimately the lignin material does degrade (Healy and Young, 1979; Benner *et al.*, 1984), albeit slowly. The reasons are discussed.

Table 4.4 Previous authors who found constant lignin quantity at individual locations.

Authors (year)	Findings
Hedges and Mann (1979b)	Found that the %OC, S/V, C/V and □ values at individual cores from the southern Washington State continental shelf and slope were all constant within the 0 to 40cm depth and with no consistent change with depth and age, indicating stable lignin components for 400 years.
Hedges *et al.* (1982)	Found that above the 8m (of a 11m core) depth of a Late Quaternary sediment core, the □ values were uniform and the constant (Ad/Al)v and (Ad/Al)s ratios for the entire core of 11m is evidence that *in situ* oxidative degradation of lignin had not occurred to an appreciable extent for the past 13000 years.
Hedges and van Green (1982)	Observed uniform lignin concentrations and compositions within individual sediment cores from the Gulf of Mexico and Washington coast.
Hedges *et al.* (1985)	Found that the buried spruce wood from a 2500-year old deposit was unaltered whereas an alder wood from the same horizon and an oak from the sediment were greatly degraded. They observed the following order of stability for the major biochemical constituents of both buried hardwoods: vanillyl and p-hydroxyl lignin structural units > syringyl lignin structural units > pectin > α-cellulose > hemicelluloses.
Ishiwatari and Uzaki (1987)	Lignin was found throughout the upper 700m of a 1400m sediment core of Lake Biwa indicating the lignin has been stable for over 0.6 million years.
Hedges *et al.* (1988a)	Found compositionally uniform sediment core over the entire 50cm length.
Miltner and Emeis (2001)	Individual locations in the Baltic Sea showed constant lignin content.
Kastner and Goni (2003)	The Amazon deep sea fan showed remarkable constancy in organic matter component.

Implications

1. There was a constant presence of lignin, indicating a constant presence of terrestrial organic matter in Loch Creran. There are several possible explanations for this.

(i) There was a continuous input of terrestrial materials from River Creran draining the catchment areas into the loch. If there was no continuous input of terrestrial materials from the river into the loch, then the highest lignin abundance would not be

found in the stations nearest the river input, as most terrestrial materials would have been transported and accumulated further down the loch.

(ii) However, if there were a continual (intermittent or seasonal) input of terrestrial material into the loch, a similar phenomenon (lignin content in sediments decreasing further down the loch) would also have been observed. Hence, the question arising is whether there was a 'continuous' or 'continual but intermittent' input of terrestrial organic matter into the loch?

From the monthly total lignin data in the sediment samples (Table 3.2), the phenomenon of intermittent input of terrestrial organic matter, if occurring, was nominal in the sediment traps, LC0 and LC1. In the sediment traps, there was a slight increase in total lignin content during February and March 2002, and during other months the total lignin was around 0.2 to 0.3mg/g. At LC1, there was a decreased in total lignin in May 2002 to 0.03mg/g, but in other months the total lignin was around 0.2 to 0.3mg/g. At LC0, total lignin was around 0.2 to 0.3mg/g for the whole sampling year.

For the locations further down the loch, at LC2, LC3, LC5 and LC6, the intermittent effect is more distinct, as seen from the greater differences in total lignin between the subsequent sampling months (Table 3.2). In Loch Etive however, these effects could not be determined as sampling was carried out for only three months (Table 3.6).

(iii) Another possibility is that there was intermittent input of lignin materials into the loch, but as the lignin material was quite stable to degradation, there was not much change in the lignin content at individual locations throughout the year.

Such intermittent input is the most likely scenario. During sampling trips to River Creran, it was observed that there were times when the river was deep and the flow swift (these were most likely times of high terrestrial materials input into Loch

Creran). There were also times when the river was shallow, with the slow flow (these were most likely periods of low terrestrial materials input into the loch).

(iv) There were no significant changes at LC0, LC1 and sediment trap at the upper most basin. For locations in other basins, there were significant differences. The most probable reason for this is due to the entrainment of materials by the inflowing water, and subsequent deposition of this material. The entrainment process, coupled with the stability of lignin material, results in constant presence of lignin material in locations in the upper most basin of Loch Creran.

2. There was no significant difference between the lignin concentration and the MET results consisting of air temperatures, rainfall and wind speed (Section 3.2.1.1). Detailed studies show that the high rainfall in February and June 2002 (Figure 3.1c) results in high sedimentation rates in April and August 2002 (Figure 3.3). High sedimentation rate in turns, results in high lignin content in the sediment trap in March and July 2002 (Figure 3.2a), and at LC1 March and June 2002 (Figure 3.2b). Hence although there was no significant correlation (regression analysis: $p>0.05$) with the seasonal parameters and sedimentation rates, the lignin abundance was affected, albeit slightly, by the rainfall and sedimentation rate.

3. The constant (Ad/Al)v values indicate that degradation of the lignin component had occurred whilst in the soils and during transportation in the water column (see also previous explanation in Section 4.2.1.3, Implication 3).

4. The (Ad/Al)v ratios were at times higher in the 0-1cm sediment layer compared to the 9-10cm sediment layers, and at times *vice versa*. The more degraded materials being deposited in the surface 0-1cm sediment could be due to sinking of some suspended particles, and from particle reworking due to bioturbation. The trapped 'fresher' materials are likely due to rapid sedimentation rate.

4.2.2.2 Transects of the lochs

Along transects of both lochs, total lignin was found to decrease significantly from head to mouth (Sections 3.2.1.3 and 3.2.2; and Table 4.1). The mean total lignin from individual locations in Loch Creran decreased from the head to the mouth and outside the loch (Table 3.5): LC0 (0.3305mg/g), LC1 (0.2180mg/g), sediment trap (0.3041mg/g), LC2 (0.1760mg/g), LC3 (0.0682mg/g), LC5 (0.1016mg/g) and LC6 (0.0470mg/g). Similar observations were also made for Loch Etive (Table 3.6): RE2 (total lignin = 0.5477mg/g), RE5 (0.4509mg/g), RE6 (0.4823mg/g) and Camas Nathais (0.0718mg/g). The Λ values also decreased from the head to the mouth of the lochs. Overall RE2, RE5 and RE6 have greater total lignin abundances than LC0, LC1 and the sediment trap samples, due to greater ratio of the catchment to the loch area for Loch Etive (see Section 4.6.2.1)

Similar circumstances, the highest lignin content was found nearest the freshwater input, and the decrease in the lignin content further offshore, were also observed in other locations from previous studies, and these are given in Table 4.5.

Table 4.5 Previous authors who also discovered that lignin decreased offshore.

Author (year)	Findings
Pocklington (1976)	Found that lignin decreased from the fjords to offshore. The lignin concentration in the Corner Brook Harbour was more than 5.0mg/g followed by St. Lawrence estuary and inshore sediments (5μg-5mg) and in the Laurentian Channel and Esquiman Channel, along the Laurentian Through to the Scotian Shelf, the lignin content was low and undetectable (<5μg).
Hedges and Parker (1976)	The offshore decrease in land-derived OM measured for these sediments probably results as the relatively resistant biological materials from land are mixed with the remains of marine organisms and deposited in progressively decreasing concentrations at the more seaward sites.
Hedges and Mann (1979b)	Found the highest lignin concentrations occurred near the mouth of the Columbia River (Λ = 6.8±1.7) and the lowest lignin concentration occurred furthest from the river mouth (Λ = 0.23±0.47), hence preferential accumulation of sedimentary lignin near the river mouth and within the mid-shelf.
Miltner and Emeis (2001)	Found that lignin contents differed significantly between individual basins, indicating that there was limited transport of terrestrial organic matter across the basin boundaries hence the sills act as barriers for particles carrying terrestrial organic matter. Pronounced compositional differences between basins indicated that inter-basin transport of terrestrial organic matter is less important than direct river input. They found that the interbasin differences in lignin content reflect the basin size, its distance from land, and the influence of rivers. For example the Gulf of Finland has a high lignin yield, receiving discharge from the River Neva, and Gdansk Bay receives water from the Vistula River.
Bianchi *et al.* (2002)	Found that lignin decreased further offshore from the lower Mississippi River (Λ =1.78) to the Louisiana shelf (Λ =0.54).

Implications

1. The decrease of lignin materials from the head to the mouth and outside the lochs signifies the importance of the rivers in transporting terrestrial materials into the lochs: River Creran at the head of Loch Creran and River Etive at the head of Loch Etive (Sections 1.3.2 and 2.1). These terrestrial materials accumulated near the river sources, hence the decrease in concentrations further down the lochs (Section 3.2.1.3: Figures 3.5 and 3.6a). Regions with high lignin yields (LC1 and LC0 in Loch Creran and RE2 in Loch Etive) are clearly distinguished from those with low yields (LC6 and Camas

Nathais). For Loch Etive, some of the lignin phenols were higher at RE6, most probably due to contribution from River Awe (Figure 3.7).

The Baltic Sea consists of several basins such as the Gulf of Bothnia, Gulf of Finland, Gulf of Riga and Gdansk Bay with freshwater input. These basins all drain into the Gotland Sea, and this leads to the ocean via the Kattegat and Skagerrak. The well-compartmentalized Baltic Sea offers a good opportunity to follow the basin-to-basin transport of terrestrial organic matter without the risk of the biomarker degradation during transport (Miltner and Emeis, 2001).

Similar circumstances also occur in lochs Creran and Etive. Both are well compartmentalized and both have freshwater input at the head of the lochs. There are two main inputs in Loch Etive, the River Etive and River Awe. Moreover, both lochs consist of just one straight route of one basin leading to the other, hence very convenient to study the input and output of terrestrial organic matter.

2. While being transported from River Creran across the loch, some of these terrestrial materials sediment out, resulting in decreasing concentrations further from the main river source.

3. The high (Ad/Al)v values of these sediments indicate that all sedimentary lignin have been subjected to a high degree of oxidative degradation and were already quite degraded. Hence further down the lochs, the lignin materials did not undergo much degradation. The mean (Ad/Al)v value for individual location in Loch Creran is as follow: sediment trap (2.68), LC0 (1.07), LC1 (0.83), LC2 (0.96), LC3 (1.19), LC5 (0.52), and LC6 (0.90) (Table 3.5). And for Loch Etive, the mean (Ad/Al)v values are as follow: RE2 (0.74), RE5 (0.72), RE6 (0.88) and Camas Nathais (0.52) (Table 3.6).

4. Besides, further down the loch, this terrestrial organic matter had been diluted with organic material of marine origin. The more enriched $\delta^{13}C$ values further down the lochs are further evidence of the mixing of terrestrial materials with marine organic matter.

5. There are several types of transport mechanisms in the lochs: vertical, advective and transportation due to gravity currents. Vertical transport is the down-column flux of materials to the surface sediments. Advection is due to the movement of water from one point to the other. This transport may occur in a horizontal direction along the major current system and in vertical direction during upwelling or sinking (Chester, 2000). Advective transport may also occur due to entrainment of the less dense freshwater with the denser saline water (Aure and Stigebrandt, 1989).

   Gravity currents transport materials by slides, slumps and gravity (Chester, 2000); this most probably transported materials from one location to another within the similar basin due to the slope or depth differences between these locations. This is seen in Loch Creran due to the decrease of the organic matter content from LC0 (approximately 15m deep) to LC1 (37m) in the same basin, and the decrease of the organic matter content from LC2 (27m) and LC3 (49m) in the same basin. The decreases of lignin, percentage organic matter due to loss on ignition and %TC and %TN from LC0 to LC1, and from LC2 to LC3, are summarized in Table 4.6.

Table 4.6 The lignin, percentage organic matter due to loss on ignition, %TC and %TN.

Parameters	LC0	LC1	LC2	LC3
Lignin	0.33	0.22	0.18	0.07
% labile OM	9.83	9.34	6.61	3.47
% refrac OM	7.28	6.88	6.15	4.91
%TOM	17.11	16.19	12.76	8.38
%TC	5.10	4.57	3.58	2.67
%TN	0.52	0.47	0.38	0.28
%TOM	4.77	4.01	3.14	1.83

6. Wangersky (1978) believed that the compression and intensification of the carbon cycle are processes that make the coastal area productive rather than the influence of the adjacent landmasses. Here is what he proposed. For a large area of the near shore environments the sea floor is within the euphotic zone, so that turbulence induced by severe storms can recirculate materials from deeper waters back into the zone of primary production. And if the water column is shallow enough, much of the particulate matter may reach the bottom before inorganic nutrients have been regenerated, and the input of nutrients into the euphotic zone may be tied to processes occurring in the sediments. Hence the whole cycle of photosynthesis, death and regeneration is compressed into a shallow zone of perhaps less than 100m deep, and is subjected to rapid turnover.

   There is no doubt that such compression occurs, especially in Loch Creran where the maximum depth is only 46m (as seen from the fresher materials being deposited in the deeper 10cm layer compared to the surface sediments). When the water column is shallow enough or the productivity of the surface water is high enough a large amount of particulate organic matter reaches the sea floor before decomposing (Wangersky, 1978). This is what had happened in Loch Creran, occasionally. When this happens, the organic content of the sediment might become so high that the dissolved oxygen content of the bottom and interstitial water cannot fully accommodate the oxidation of this organic matter to CO_2 (Wangersky, 1978). This however, does not occur in Loch Creran, as there is sufficient water circulation to ensure that sufficient oxygen is present.

   Although this "compression" conditions does seem to occur in Loch Creran due to the shallow water column and high sedimentation rates at times, contrary to the suggestion by Wangersky (1978), land-derived organic matter does exert significant effect on the biodegradability of the sediment organic matter in Loch Creran on the whole. First of all, the carbon, organic matter and lignin input near the river source are the highest and decrease further down the loch, as is the oxygen uptake rate. In fact, the terrestrial materials play an important role in fuelling the organic matter cycling in the loch, as seen

from the significant correlation between lignin with $\delta^{13}C$ values, percentage organic matter due to loss on ignition and %TC.

4.2.3 Summary

1. The Λ values in Loch Creran and Loch Etive are within or just slightly lower than the ranges of Λ values reported in other locations from previous studies such as estuaries, river, coastal and lake zones: Columbia River, Atchafalaya bay and delta regions, Washington margin continental shelf and Columbia River basin, and the Amazon basin (Hedges and Mann, 1979b; Gordon and Goni, 2003; Prahl *et al.*, 1994; Kastner and Goni, 2003). This could be due to sedimentation, and the characteristic that both lochs drain smaller catchment areas.

2. The Λ values in Loch Creran and Loch Etive were slightly higher than some other locations such as a location furthest from the Columbia River, Washington slope Holocene and Pleistocene sediment, Washington continental slope, Cascadia Bay and Cascadia sea channel, Gulf of Mexico, and Baltic Sea (Hedges and Mann, 1979b; Hedges and van Green, 1982; Prahl *et al.*, 1994; Goni *et al.*, 1997 and 1998; Miltner and Emeis, 2001); the reason being these locations were located further from the river sources.

3. Hence the high or low total lignin content is governed by the extent of catchment areas draining into the aquatic environments, and also due to the distances from the main river sources or input of terrestrial debris.

4. The higher (Ad/Al)v values in the sediments from lochs Creran and Etive are most probably due to degradation of the terrestrial organic matter prior to transportation into the lochs (Hedges *et al.*, 1986; Ertel *et al.*, 1986; Hamilton and Hedges, 1988; Hedges *et al.*, 1988; Section 1.4.3), good ventilation of the loch water, and/or because the non-woody angiosperm vegetation predominates.

5. The constant lignin content at individual locations is due to continual but intermittent input of terrestrial materials into the loch, and continual transport of these materials further down the lochs.

6. Total lignin is found to be highest at the heads of the lochs, decreasing significantly further down the lochs, indicating the importance of the contribution of terrestrial organic matter from river sources.

4.2.4 Strengths and weaknesses of past and present studies

1 Of all the studies to date, only locations from the Baltic Sea (Miltner and Emeis, 2001), the lower Mississippi River and Louisiana shelf (Bianchi *et al.*, 2002) and lochs Creran and Etive (this study) are enclosed systems so that the input and output of terrestrial materials could be investigated.

Only two locations within the lower Mississippi River were sampled that could be considered located within enclosed system.

As for the Baltic Sea, this consists of a few basins draining into the Gotland Sea (Section 4.2.2.2, Implication 1). In comparison with the Baltic Sea, lochs Creran and Etive offer a more straightforward system to study the transport of terrestrial organic matter, as there is only one route from the head to the mouth of the lochs, with the main river sources draining into the head of the lochs.

Hedges and Mann (1979b) had collected sediments from seven locations along a transect perpendicular to the coastline south of Grays Harbour, Washington. These locations were however, not within an enclosed system. Although they had found offshore decrease in land-derived organic matter, in my opinion, the enclosed system in lochs Creran and Etive provides a more reliable information of the transport of terrestrial

organic matter, as the transport of these materials width-wise across the lochs is cancelled; hence only the transport of materials along the lochs is determined.

2 In contrast to this study where sediment samples were analysed only to 10cm deep, these authors had analysed the sediment cores to depth up to 700m. Hedges and Mann (1979b) analysed eight sediment cores from 20 to 45cm, Hedges and van Green (1982) analysed four Quaternary sediment cores from the Gulf of Mexico and one core from the Washington State coast, Hedges *et al.* (1982) analysed an 11m core of the Late Quaternary sediment from the mid-basin of Lake Washington, and Ishiwatari and Uzaki (1987) analysed one vertical profile of a 700m core.

Hence the weakness in the present study lies in that only the 10cm depth of the sediments were analysed. Vertical profile of lignin for the whole 0 to 10cm sediment depth was studied for sediment collected from LC1 on 4.6.2002 (Section 3.2.1.2; Table 3.4). However, the strength of this study lies in that there were a total of nine samples where the 0-1cm and 9-10cm sediment layers were studied (Section 3.2.1; Table 3.2).

3 To date it is known that angiosperm tissues are more prone to degradation compared to their counterpart the gymnosperm tissues (Section 4.2.1.3, Implication 4). However, it is not known whether this will also result in an overall higher vanillic acid to vanillin ratio in the angiosperm compared to gymnosperm tissues.

4.3 VEGETATION SOURCES

4.3.1 S/V and C/V ratios

Lignin-derived phenols can be divided into three groups: vanillyl, syringyl and cinammyl phenols (Section 1.5.1.5). Angiosperm lignin contains both the syringyl and vanillyl groups, hence S/V>0, and the S/V reflects the contribution of angiosperm tissues; gymnosperm produces only vanillyl phenols; and only non-woody tissues produce cinnamyl phenols, hence the C/V ratio is used to distinguish between woody and non-woody tissues (Leo and Barghoorn, 1970; Sarkanen and Ludwig, 1971; Hedges and Mann, 1979a; Hedges *et al.*, 1982; Miltner and Emeis, 2001). According to Hedges and Parker (1976), angiosperm wood has an S/V of 3/1 to 5/1, and non-woody angiosperm tissues have an S/V ratio of less than 3. Low S/V ratios indicate the presence of gymnosperm tissues due to the presence of the vanillyl groups, and high S/V and C/V ratios (exceeding 0.4 and 0.15) indicate the presence of non-woody angiosperm tissues (Goni *et al.*, 2000). In order to make clearer understanding of these lignin parameters, a table of the plant tissues representative is constructed (Table 4.7).

The S/V values in Loch Creran ranged from 0.16 to 1.61 (Section 3.2.1.3, Table 3.5), and in Loch Etive, from 0.81 to 1.03 (Section 3.2.2, Table 3.6), indicating that angiosperm tissues predominate in both environments. The C/V ratios in Loch Creran range from 0 to 1.47, and in Loch Etive from 0.29 to 0.57. Only values from LC1 to LC5 in Loch Creran, and values from RE2 to RE6 in Loch Etive are used to derive these ranges, yet the values from LC6 and Camas Nathais also fall within these ranges. These C/V values are about the same as those obtained from the Gulf of Mexico sediments (0.28-0.83; Goni *et al.*, 1997), indicating the presence of non-woody tissues. Hence the S/V and C/V ratios in both lochs indicate the presence of non-woody angiosperms, and this is confirmed with the lignin phenol compositional plots (see Section 4.3.2).

Table 4.7 Classes of vascular plant materials. G = woody gymnosperm; g = non-woody gymnosperm; A = woody angiosperm; a = non-woody angiosperm; M = possibly marine phytoplankton.

Lignin group	Lignin phenol	Vegetation type				
		G	g	A	a	M
P	p-hydroxybenzaldehyde	√	√		√	√
	p-hydroxyacetophenone	√	√		√	√
	p-hydroxybenzoic acid	√	√		√	√
V	Vanillin	√	√	√	√	
	Acetovanillone	√	√	√	√	
	Vanillic acid	√	√	√	√	
S	Syringaldehyde			√	√	
	Acetosyringone			√	√	
	Syringic acid			√	√	
C	p-coumaric acid		√		√	
	Ferulic acid		√		√	

4.3.1.1 S/V ratios

The S/V ratios are constant at individual locations, but decreased slightly from the head to the mouth of the lochs.

Implications

1. In Loch Creran, the S/V ratios at LC1 (Section 3.2.1.1, Table 3.2b; Figure 4.2a) were quite constant throughout the year. There were no significant differences (ANOVA: $p>0.05$) in the S/V values apart from the increase in February and decrease in August 2002 (there is no significant differences in these increase and decrease). Even then, these increases were not statistically significant (ANOVA: $p>0.05$; Table 3.2b). The S/V ratios in the sediment trap samples (Section 3.2.1.1, Table 3.2a; Figure 4.2b) were also constant apart from the non significant increase (ANOVA: $p>0.05$) in May 2002. This could be due to the occasional local effect such as lumbering.

   The constant S/V ratios of about 0.6 throughout the year have several implications. The most probable reason would be the same reason for the constant lignin concentration at individual locations (Section 4.2.2.1): continual but intermittent input of lignin materials into the loch. Also, there was constant transport of materials down the loch. Further, as most lignin materials in the lochs are degraded, this results in stable S/V ratios at LC1 throughout the year.

2. Also, both the syringyl and vanillyl phenols constitute the total lignin content, as evidenced from the significant strong positive correlations between total lignin with the vanillyl, syringyl and cinnamyl phenols (as shown in Table 3.3 and the results therein). Hence the constant S/V ratios at individual locations indicate the same source of vegetation input into Loch Creran.

3. As the V, S and C constitute of the total lignin, and there are strong correlations between these groups (Table 3.3), the increase of total lignin also corresponds to the increase of

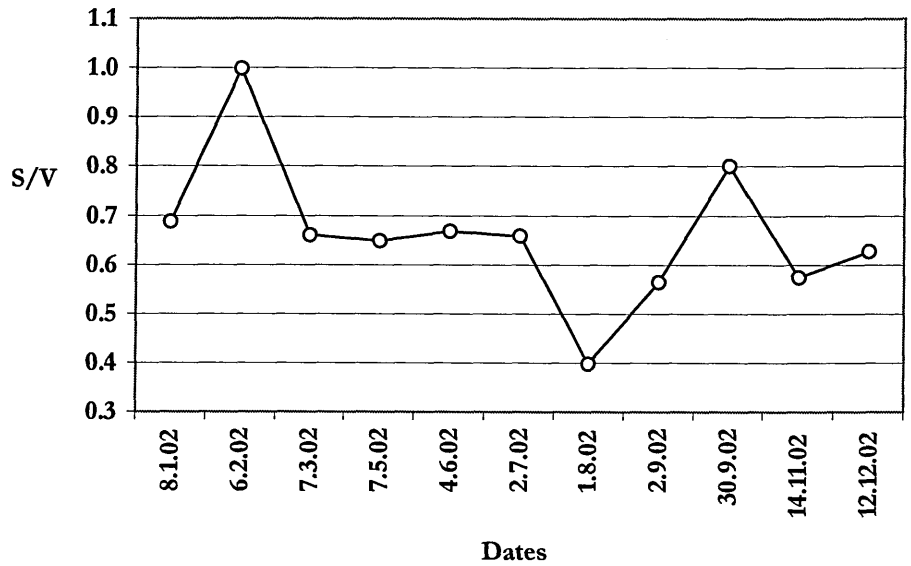
these lignin phenol groups. Hence the V, S and C contents were also due to rainfall and sedimentation rate, as explained in the previous chapter (Section 4.2.2.1: Implication 2).

4. Along transect of Loch Creran (Figure 4.2c) the mean value of S/V for LC0 is 0.6297, LC1 0.7026, sediment trap 0.7631, LC2 0.5394, LC3 0.4988, LC5 0.4169 and LC6 0.3992. Data analysis using single factor ANOVA shows significant differences ($p<0.05$) in the S/V ratios from the head to the mouth of Loch Creran. The S/V ratio decreased from LC0 to LC6 by a factor of 1.58. By using the mean S and V values, both decreased by factors of 12.56 and 7.17 respectively, from LC0 to LC6. There is greater decrease of the syringyl compared to the vanillyl phenol, as the latter is more stable to degradation (Hedges *et al.*, 1985). Hence the decrease of the S/V ratios from the head to the mouth of the lochs indicates diagenesis of the lignin material during transport.

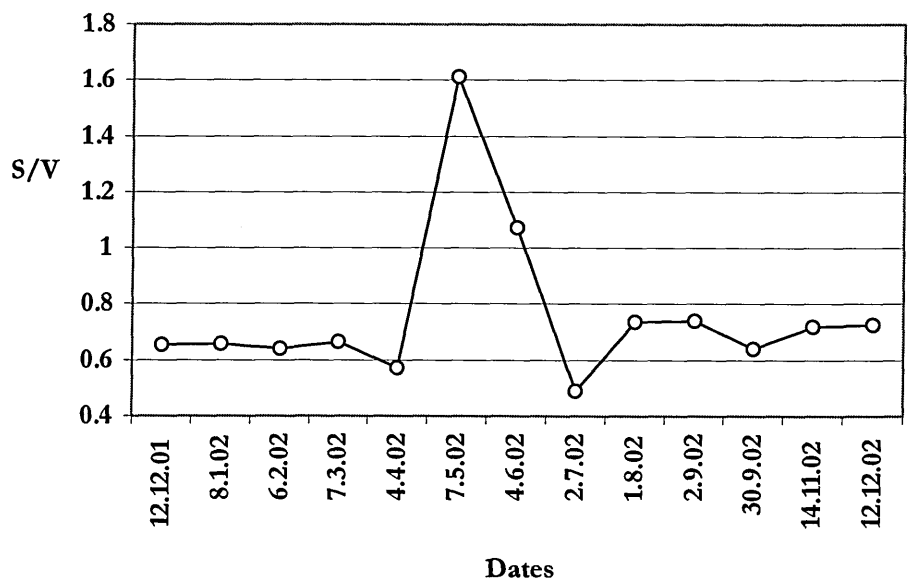
   Could the decrease of S/V ratios down Loch Creran be due to changing vegetation source rather than diagenesis? This is unlikely as the syringyl phenols decreased by a larger factor than vanillyl phenols. If there is an input of fresh vegetation, vanillyl phenols will most probably show greater change, as these are the oxidation products of both angiosperm and gymnosperm, whereas the syringyl phenols are the oxidation products of the angiosperm tissues.

5. In Loch Etive, there is significant decrease in the S/V ratios further down the loch (Figure 4.2d). The vanillyl and syringyl phenols decreased by factors of 6.40 and 10.85, respectively from RE2 to Camas Nathais. This was due to the larger decrease in the syringyl over vanillyl phenols further down the loch, most probably also because of the enhanced degradation of syringyl phenols. Hence similarly to Loch Creran, decrease in S/V ratios in Loch Etive is also due to diagenesis.

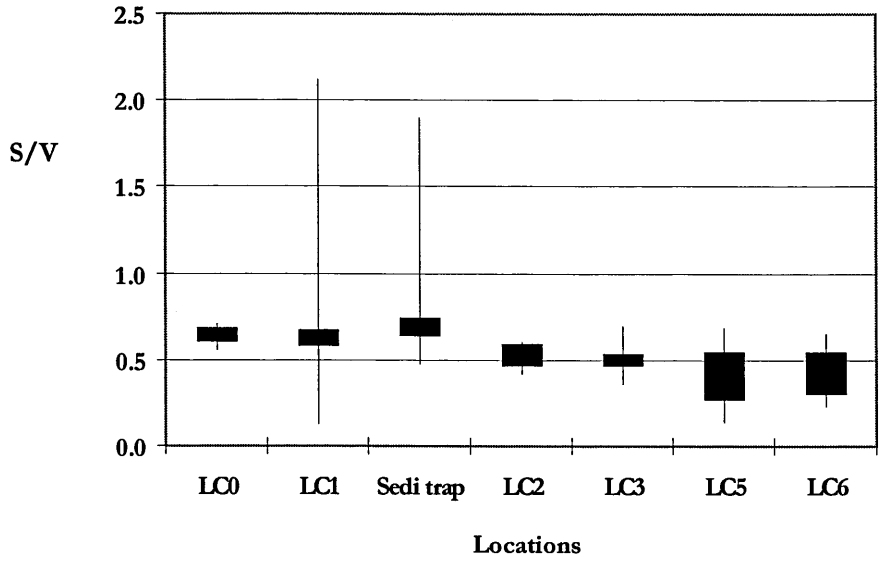
(a) LC1.



(b) Sediment traps.



(c) Loch Creran.



(d) Loch Etive.

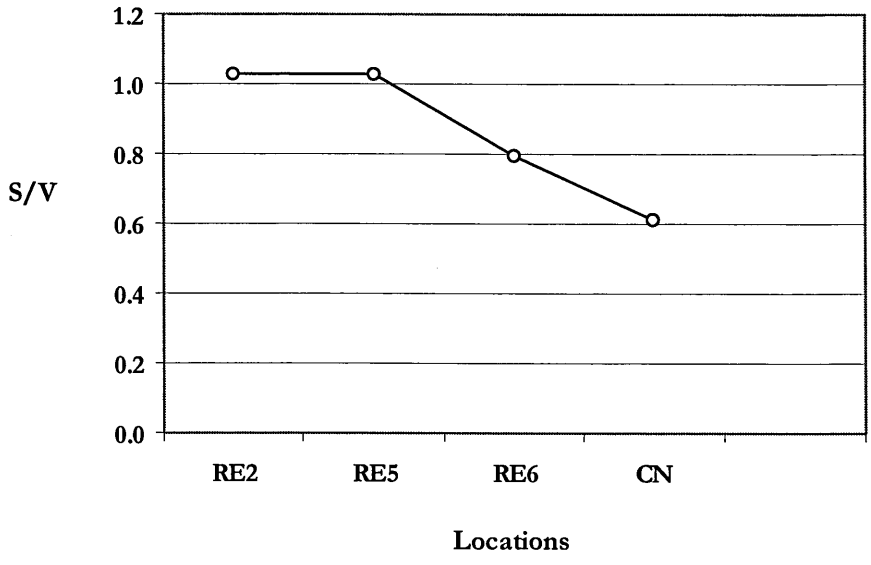


Figure 4.2 The S/V ratios for surface sediments. Plots (a), (b) and (d) consist of mean values for the sampling locations and dates. Only box plot is drawn for Loch Creran as in (c) as individual locations throughout the year consist of enough results to do this.

4.3.1.2 C/V ratios

The C/V values from Loch Creran, Loch Etive and the Gulf of Mexico are about four times greater than values reported in other studies (Table 4.2). Far greater C/V values in the Scottish sea lochs indicate the presence of a greater abundance of non-woody tissues in these lochs. C/V values were low in Lake Biwa (0.14; Ishiwatari and Uzaki, 1987) and the lowest in the Washington margin continental shelf (0.05; Prahl *et al.*, 1994), and the mouth of Columbia River (0.07; Hedges and Mann, 1979b), and the lower Mississippi River and Louisiana shelf sediments (0.03-0.10; Bianchi *et al.*, 2002).

Implications

1. C/V ratios at LC1 and sediment trap sediments (Figure 4.3 a, b) both show more fluctuation compared to the S/V ratio (Figure 4.2 a, b). Overall C/V values seem to decrease slightly from the beginning towards the end of the year, indicating the decrease of non-woody tissues with time as these were most probably being transported further down the lochs via the hydrodynamic sorting process.

   Could this also indicate an increase in gymnosperm tissues? If there was an input of gymnosperm tissue, the input was only very slight as the data presented in Section 4.3.2 indicate the presence of non-woody angiosperm tissues (Figure 4.4). Besides, the good correlations between S and V and between V and C (Table 3.3) indicate the presence of similar vegetation sources.

2. Although there is no significant difference (ANOVA; $p>0.05$) in the C/V ratios for the Loch Creran transect (this is most probably due to the wide ranges of the ratios in all locations) the C/V ratios seem to increase towards the mouth of the loch (Figure 4.2c). The C/V ratio increases from LC0 to LC6 by a factor of 1.35. This is most probably due to the hydrodynamic sorting process transporting finer sized non-woody tissues further down the loch, while retaining the more dense woody materials within the near shore

environments (Prahl, 1985; Prahl *et al.*, 1994; Goni *et al.*, 1998; Miltner and Emeis, 2000; Bianchi *et al.*, 2002).

3. Contrary to Loch Creran, the C/V ratios in Loch Etive decreased from the head to the mouth of the loch. The decrease of the C/V ratios from the head to the mouth of Loch Etive however, indicates that processes other than hydrodynamic sorting predominate. An alternative view is the input of fresh vegetation sources consisting of woody gymnosperm from the River Awe into RE6, and thence to Camas Nathais. Figure 4.7 and explanation therein indicate otherwise.

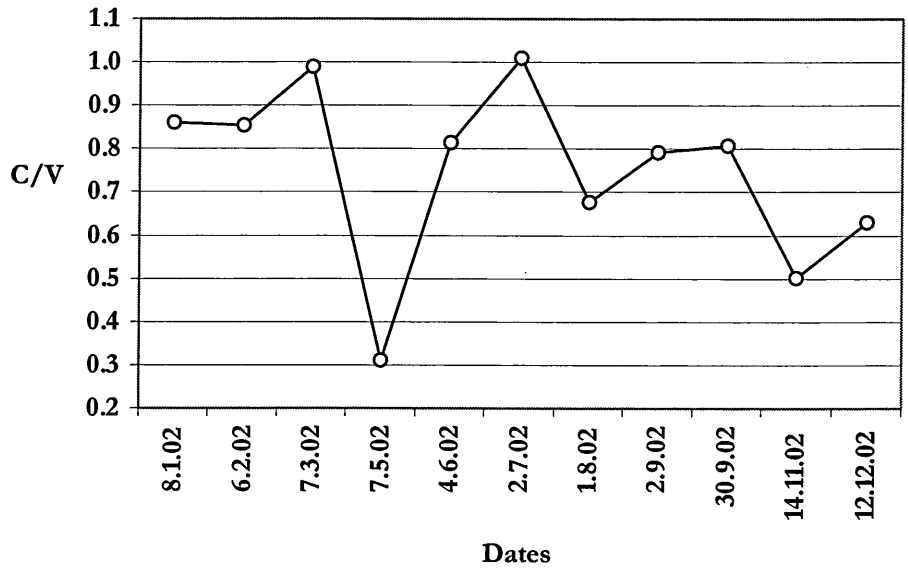
   Another reason could be that the cinnamyl phenols in Loch Etive still undergo diagenesis, as seen from the slightly lower (Ad/Al)v values for Loch Etive compared to Loch Creran sediments (Tables 3.5 and 3.6). In Loch Etive, the phenol groups decreased in this order (the % decrease of the phenol groups from RE2 to RE6 are given in parentheses): C (29.42%) > S (14.18%) > V (6.38%). As cinnamyl and syringyl phenols are more prone to degradation than the vanillyl phenols the largest percentage decrease of C followed by S and V indicates that in Loch Etive, the lignin phenols still undergo diagenesis.

   Lobbes *et al.* (2000) and Dittmar and Lara (2001) found preferential degradation of the cinnamyl phenols, and Hedges *et al.* (1986) and Ertel *et al.* (1986) found preferential degradation of syringyl compared to other phenol groups.

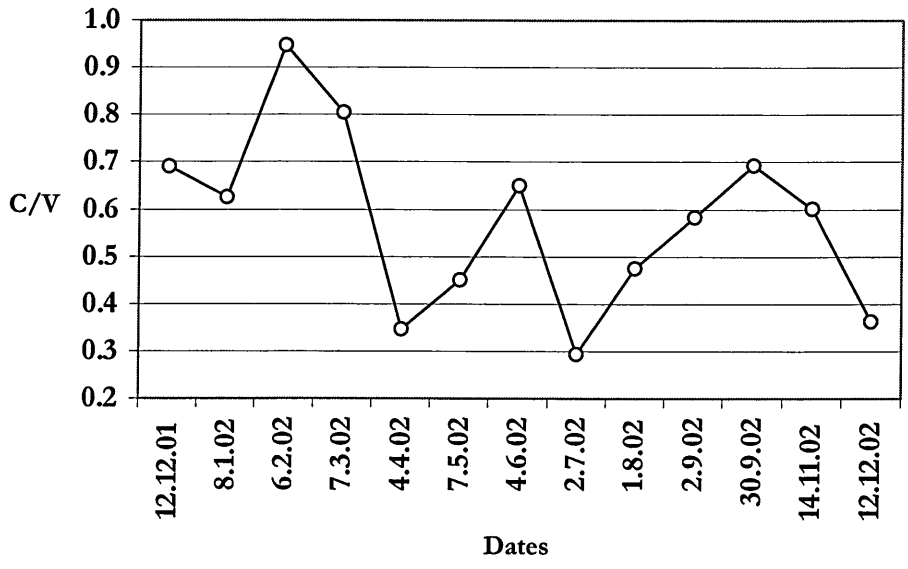
   In Loch Creran lignin phenol groups decreased in this order (the % decreases from LC0 to LC5 were given in parentheses): S (74.92%) > V (68.04%) > C (65.63%). The overall higher percentages decrease of the V, S and C phenols in Loch Creran compared to Loch Etive indicates that sediments in Loch Creran had been subjected to more organic matter degradation. One reason is that in Loch Creran, the lignin phenols are more affected by the hydrodynamic sorting process compared to diagenesis, and the better circulation of the water system and better ventilation than Etive, enabling better degradation of organic

matter in Loch Creran. This is also confirmed by the slightly higher (Ad/Al)v values from Loch Creran sediments compared to sediments from Loch Etive (Tables 3.5 and 3.6). Overall the differences in stability of lignin phenol groups between Loch Creran and Loch Etive is due to differences in the hydrodynamic and hydrological regimes between the two lochs (see Section 4.6).

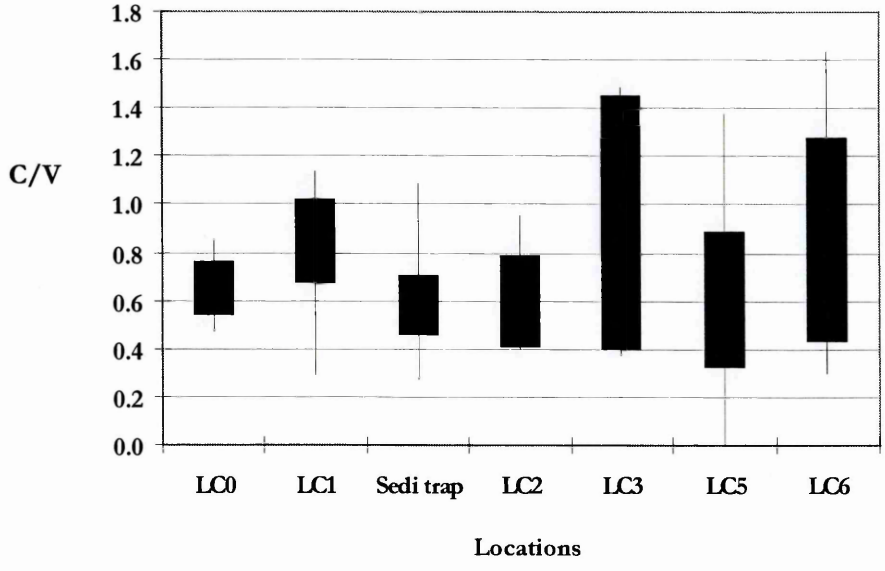
(a) LC1.



(b) Sediment traps.



(c) Transect of Loch Creran.



(d) Transect of Loch Etive.

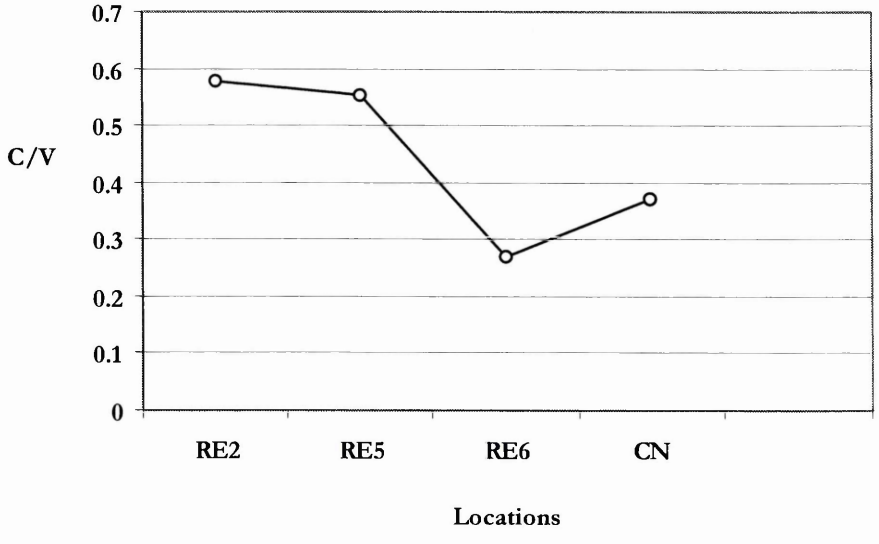


Figure 4.3 The C/V ratios for surface sediments. Enough data from individual locations for Loch Creran transect enable box plot to be drawn as in (c). For LC1, sediment trap and Loch Etive results, the mean values are used: (a), (b) and (d).

4.3.2 Lignin compositional plots

Lignin compositional plots consisting of S/V versus C/V, S versus C, S versus V, and C versus V were firstly used by Hedges and Mann (1979a) in order to distinguish between plant types. The authors had subjected different plant tissues to CuO oxidation and the lignin phenols obtained were used to derive these plots. Hedges and Mann (1979b) used these plots to derive plant sources from lignin phenol yields in sediment samples. Since then lignin compositional plots have been widely used by many to derive vegetation sources from lignin in sediment samples (Table 4.2). According to Miltner and Emeis (2000), as diagenesis of terrestrial organic matter is weak and is similar at all stations, it was assumed that the S/V and C/V ratios were not greatly affected, and if so, to a similar extent. Hence they can be used to characterize the origin of the terrestrial organic matter. Similar circumstances apply here. Both the S/V and C/V ratios in lochs Creran and Etive are constant at individual locations; hence they can be used to determine the vegetation sources.

Figure 4.4 gives lignin compositional plots for LC1 sediment samples. In plot (a), points along C/V=0 would represent both gymnosperm and angiosperm wood, as p-coumaric and ferulic acids are not produced by woody tissues. Points along S/V=0 would represent gymnosperm tissues because they do not produce syingyl phenols (hence S=0). The point where S/V=0 and C/V=0 indicate the presence of woody gymnosperm. Hence the points scattered in the middle of the S/V versus C/V diagram indicate the presence of non-woody angiosperm tissues. For plot (b) of S versus C, the points along C=0 would indicate the presence of woody tissues. Points along S=0 would indicate the presence gymnosperm tissues as gymnosperms do not produce syringyl phenols. The point where S=0 and C=0 would represent the presence of gymnosperm wood. Hence the points scattered in plot (b) indicate the presence of non-woody angiosperm tissues. There is one point with very low S and C due to very low lignin content for that month (May 2002: Table 3.2b). In plot (c) of S versus V, the line S=0 would represent the presence of gymnosperm tissues, hence the scattered points in this diagram represent the presence of

angiosperm tissues. The good correlations between S and V, and between C and V all indicate that these phenols originate from the same vegetation source.

All these plots in Figure 4.4 indicate the presence of non-woody angiosperm tissues. If gymnosperm tissue exists, the S/V ratios would be lower than the scattering around 0.6 as in plot (a), because gymnosperm produces vanillyl phenols, hence the S/V ratio would become lower. If woody tissue exists, the C value would be lower due to dilution with the cinnamyl phenols produced by non-woody tissues.

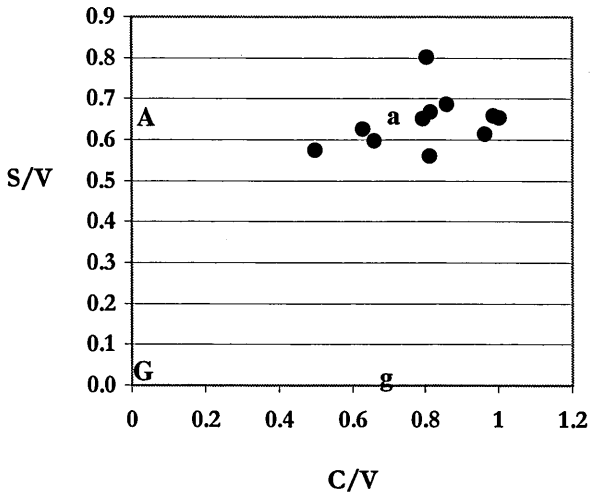
For the sediment trap samples (Figure 4.5), both S/V versus C/V and the S versus C plots (Figure 4.5 a and b) indicate the presence of non-woody angiosperm tissues. All the S, V and C phenols originate from the same vegetation source, as indicated by the good correlations between S and V, and between C and V.

For Loch Creran transect (Figure 4.6), the S/V versus C/V plot indicates the presence of non-woody angiosperm tissues. But the S versus C plot, where C=0 indicate the presence of woody tissues for one location within the loch for one month. The data within the vicinities of S/V≠0, C/V≠0, S≠0 and C≠0 all indicate the presence of non-woody angiosperm tissues. Some of the very low S, V and C values are due to the very low lignin content. The good correlations between S and C and between C and V in plots (c) and (d) indicate the same vegetation source along the loch, and also that all these phenols decrease further down the loch. Plot (b) and (d) indicate the presence of woody and angiosperm tissues. Overall, in Loch Creran the non-woody angiosperm tissues predominate, and some woody tissues exist for a short duration in one of the locations (where C=0 for Figure 4.6b, and where V=0 for Figure 4.6d) at LC5 during February 2002 (Table 3.2f).

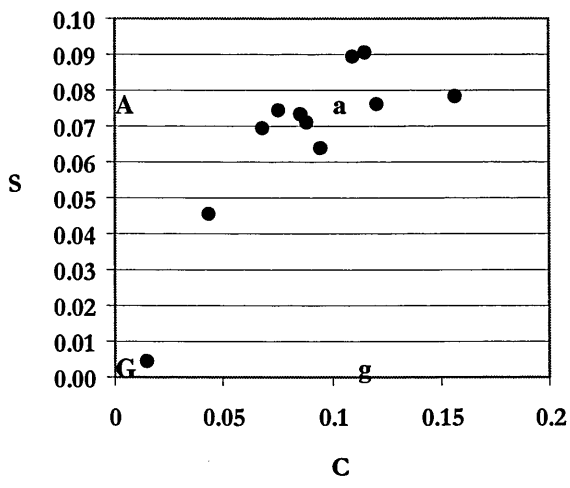
For Loch Etive (Figure 4.7), all the plots indicate the presence of non-woody angiosperm tissues. The very low S, C and V values as seen from plots (b), (c) and (d) are due to the very low lignin content at Camas Nathais. It seems that the vegetation source for RE2 and RE5 are closely

related, and the three sampling trips at RE6 gave closely related vegetation sources, indicating very constant vegetation sources at the individual locations along the loch.

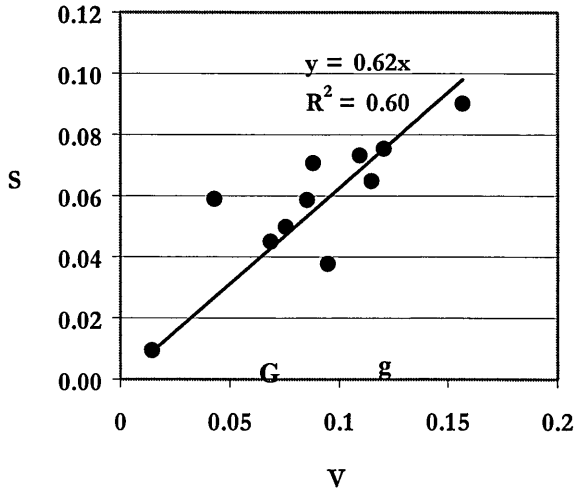
(a) S/V versus C/V



(b) S versus C



(c) S versus V



(d) C versus V

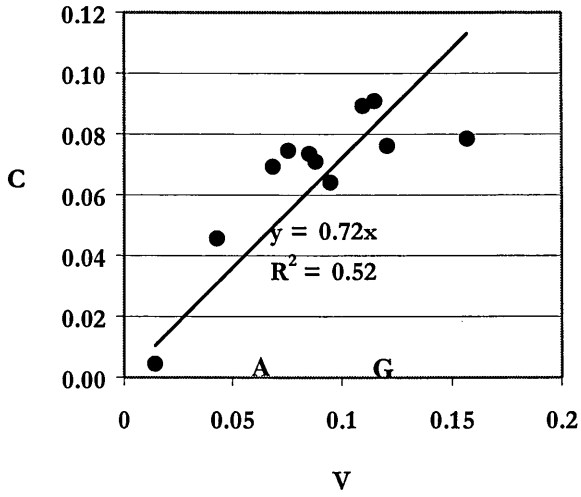
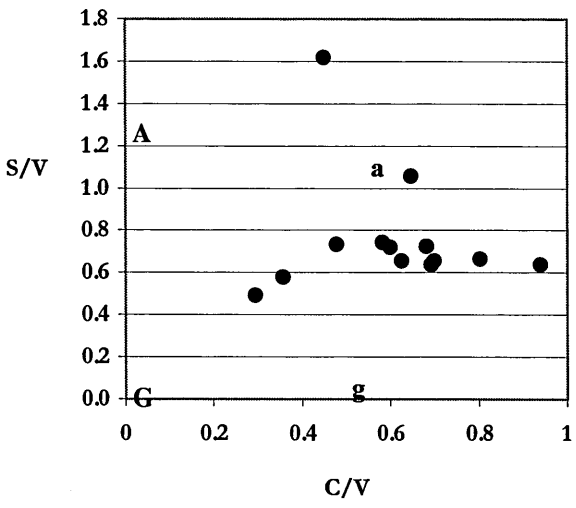
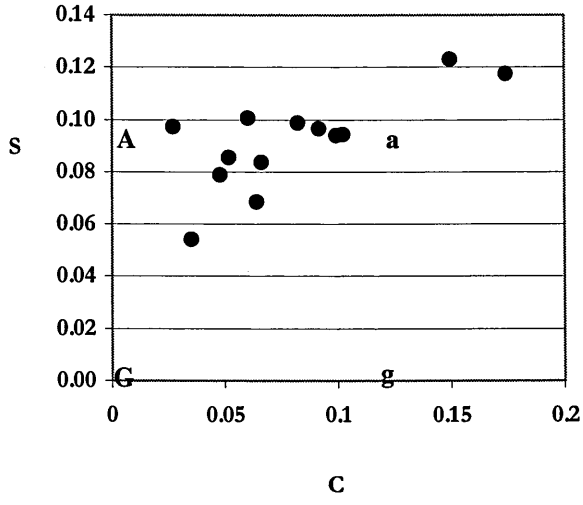


Figure 4.4 Lignin compositional plots for LC1.

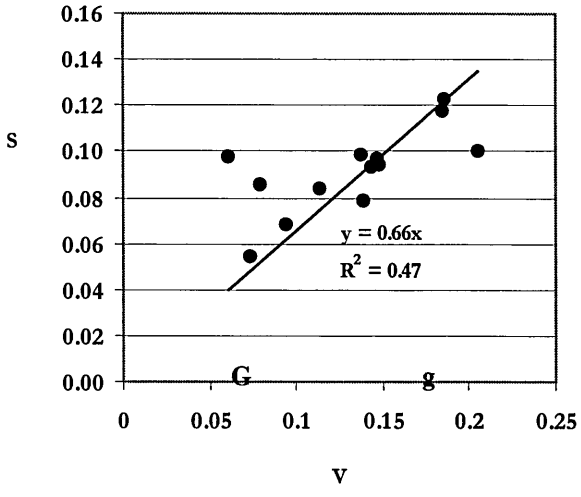
(a) S/V versus C/V



(b) S versus C



(c) S versus V



(d) C versus V

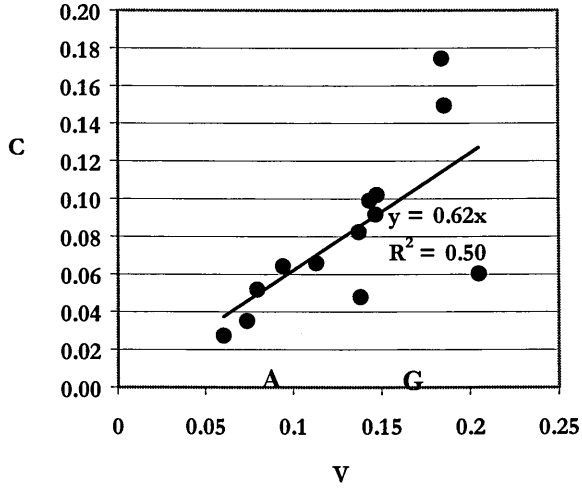
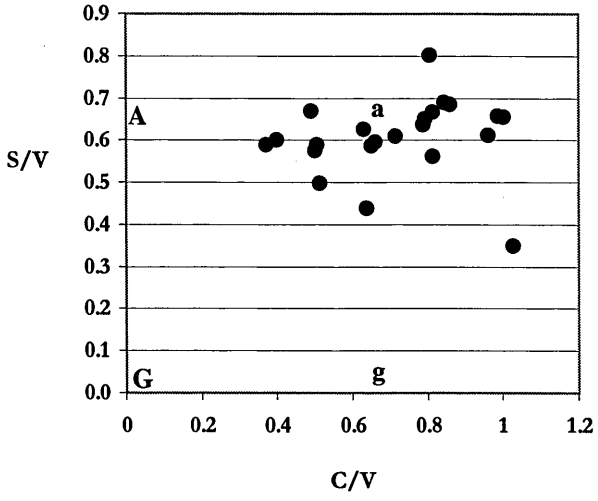


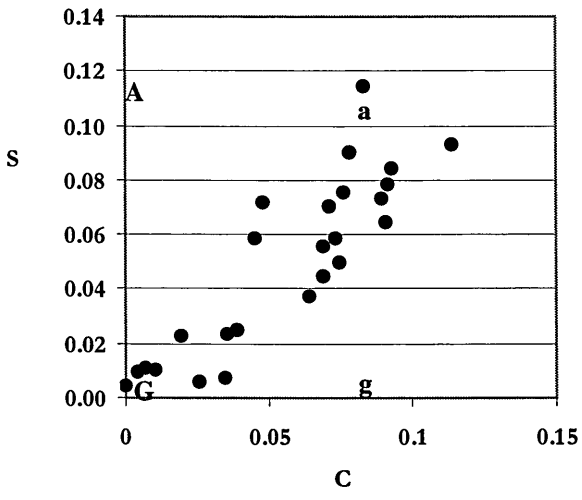
Figure 4.5 Lignin compositional plots for sediment trap samples.

A

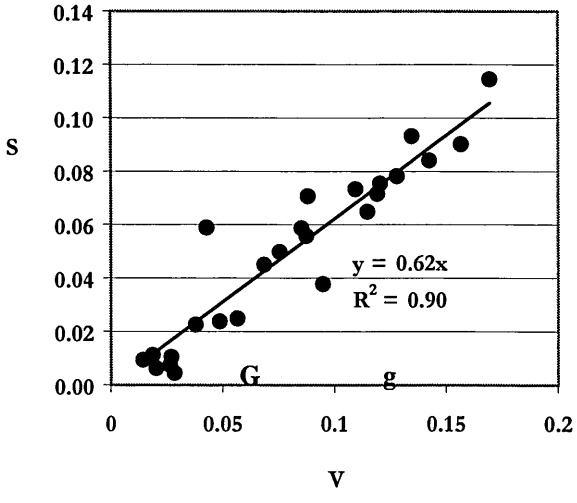
(a) S/V versus C/V



(b) S versus C



(c) S versus V



(d) C versus V

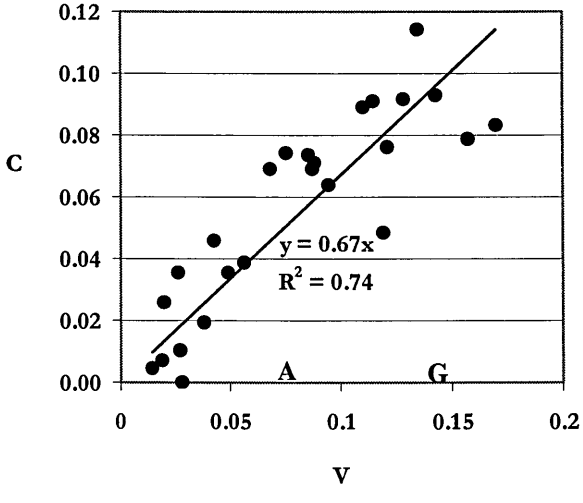
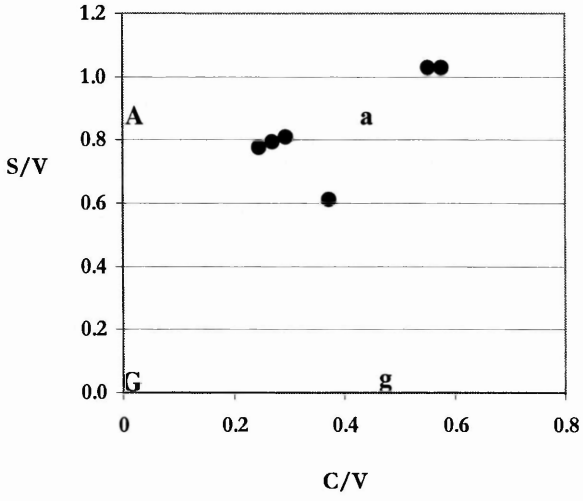
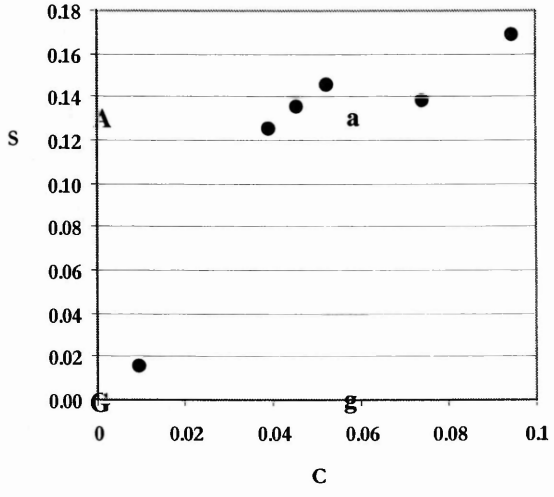


Figure 4.6 Lignin compositional plots for Loch Creran transect.

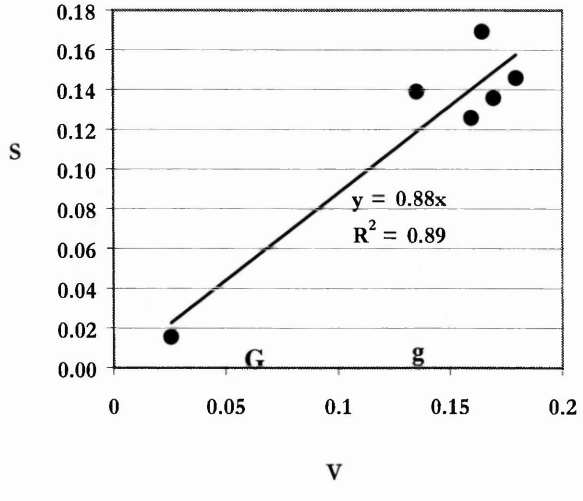
(a) S/V versus C/V



(b) S versus C



(c) S versus V



(d) C versus V

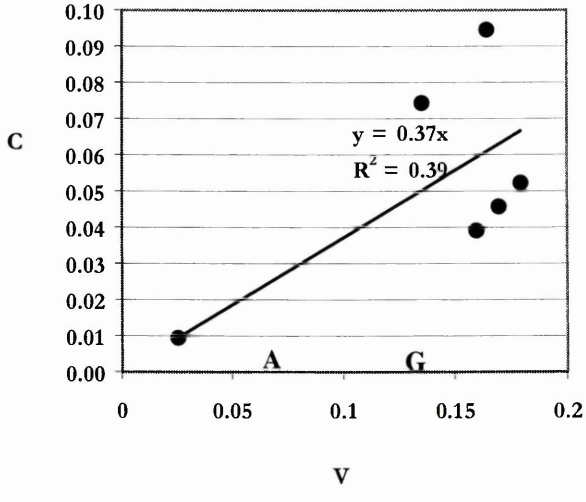


Figure 4.7 Lignin compositional plots for Loch Etive transect. Abbreviations: A – angiosperm woods; a – non-woody angiosperm tissues; G – gymnosperm woods; g – non-woody gymnosperm tissues. "A" occurs where C/V=0 and C=0; "G" occurs where S/V=0, C/V=0, S=0, C=0; "g" occurs where S/V=0, S=0; "a" occurs where S/V≠0, C/V≠0, S≠0, V≠0, C≠0. For further information, see text (Section 4.3.2).

Implications

1. S/V is the ratio of syringyl to vanillyl phenols. The vanillyl phenols could be due to the mixture of angiosperm and gymnosperm, and the S/V ratios will still exceed 0. Hence there was no limit of the S/V ratios that indicate angiosperm or gymnosperm tissues. Previous studies show that low S/V ratios indicate the presence of gymnosperm tissues (Table 4.2). The S/V ratios obtained in this study were around 0.6, indicating the presence of angiosperm tissues.

2. Similar explanation also applies for the C/V ratios. Although woody tissues do not produce cinnamyl phenols, the C≠0 could also be due to a mixture of non-woody and woody tissues. Comparison with past studies, however, indicates the high C/V ratios in lochs Creran and Etive show the presence of non-woody tissues.

   If there were woody tissues present, all the vegetation sources were very well mixed. Figure 4.6 (b) shows the presence of woody tissues at LC5 in Loch Creran.

4.3.3 Vegetation maps

Having confirmed the vegetation sources in both lochs Creran and Etive using the lignin compositional plots (Section 4.3.2), vegetation sources in both lochs were further confirmed by aerial photographs obtained from the Scottish Natural Heritage, Oban (Figure 4.8). These aerial photographs show the vegetation patches surrounding the Loch Creran consist of deciduous plants. Some of these are surrounded by spruce on the higher grounds. These pictures were also confirmed by personal observations during the sampling trips.

All these parameters: percentages labile, refractory and total organic matter due to loss on ignition, TC, TOC, TN, and phosphate content decrease from the head to the mouth of the lochs. The $\delta^{13}C$ values increase further down the loch, signifying the presence of terrestrial organic matter at the head of the loch; further down the loch, marine organic matter predominates. All these also indicate that the terrestrial materials and carbon sources into the lochs originate from the river sources, and not from the vegetation sources surrounding the lochs.

Observation from the Ordnance Survey vegetation map (Steven J. Gontarek, *pers. comm.*), shows that the catchment of both lochs consists of both angiosperm and gymnosperm plants. Hence the detection of non-woody angiosperm tissues in both lochs is because the deciduous plants occupy the land in the vicinity surrounding the rivers and lochs, and they shed their leaves annually. Some gymnosperms are evergreen. Some shed their needles, but these are usually found on the higher grounds. Hence most of the vegetation sources transported into the lochs are from non-woody angiosperm tissues.

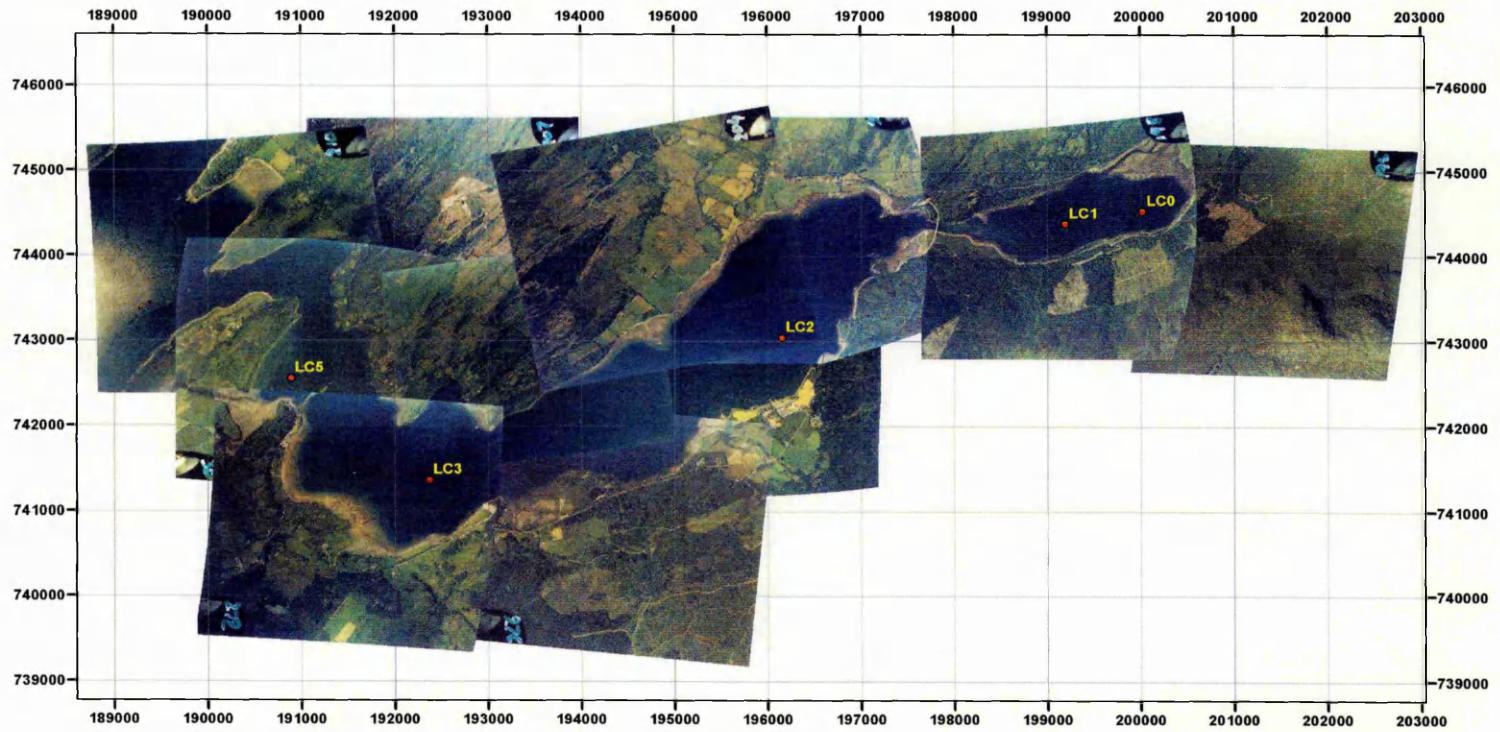


Figure 4.8 Loch Creran aerial photograph. The aerial photographs were obtained from the Scottish Natural Heritage, Oban. These were then pieced together by Steven Gontarek (Dunstaffnage Marine Laboratory).

4.3.4 Plant samples

4.3.4.1 Lignin

Different plant samples were subjected to CuO oxidation and the results are given in Table 4.6. Detection of lignin in plants confirmed the applicability of the CuO oxidation process in the elucidation and quantification of lignin phenols. The concentrations ranges reported by Hedges and Mann (1979) for these vegetation types are given: gymnosperm tissues' p-coumaric acid range 3.3-5.7mg/g of sample, gymnosperm tissues' ferulic acid 0.7-1.8mg/g, angiosperm tissues' p-coumaric acid 1.4-6.6mg/g and angiosperm tissues' ferulic acid 0-7mg/g. In this study, the concentrations obtained for the plant samples are within these ranges.

Only few authors who have analysed original plant materials for their lignin oxidation products: Hedges and Parker (1976), Hedges and Mann (1979a) and Wilson *et al.* (1985). Hedges and Parker (1976) indicated their results in % composition. In Table 4.8, individual lignin phenols (also in % composition) are also summed into their respective groups: P, V, S, and C, and the S/V, C/V and (Ad/Al)v ratios derived. For the samples where the % composition does not total up to 100%, other oxidation products were present, for example 3,5-dihydroxybenzoic acid.

Hedges and Parker (1976) found that gymnosperm tissues do not produce syringyl and cinnamyl phenols, they produce just vanillyl and p-hydroxyl phenols, woody angiosperms produced syringyl and vanillyl phenols and non-woody angiosperm tissues produce all the p-hydroxyl, vanillyl, syringyl and cinnamyl phenols. Wilson *et al.* (1985) observed higher concentrations of syringyl and vanillyl phenols in woody angiosperms than non-woody tissues, the needles, but the non-woody tissues (needles) have higher concentration of cinnamyl phenols than the woody tissues (branches) (Table 4.8).

Plant samples collected for this study were a mixture of fresh and old shed leaves. However, all were analysed about a year after collection, hence all could be considered 'old'. Both Hedges and Mann (1979a) and Wilson *et al.* (1985) analysed samples of leaves and barks from growing

trees during late summer and spring. This is most probably the reason for the slightly higher (Ad/Al)v values for the plants analysed in this study.

The angiosperm tissues used in this work were oak and birch. The total lignin, however, was higher in the leaves than stem samples. All the plant samples analysed in this study were found to produce all the lignin phenols: p-hydroxyl, vanillyl, syringyl and cinnamyl phenols. This could be due to the incomplete separation between the stems and leaves. One distinct observation is that oak, birch and bracken leaves all have greater C/V ratios than their stems, as non-woody tissues produce more cinnamyl phenols, hence they have greater C/V values than woody tissues. Also, all leaf samples have higher (Ad/Al)v values than stems.

Compared with results from plant samples, S/V and C/V ratios from sediments in both lochs Creran (S/V=0.16-0.61; C/V=0-1.47 for sediment samples) and Etive (S/V=0.81-1.03; C/V=0.29-0.57 for sediment samples) indicate the presence of all the plants analysed except spruce and oak leaves. As the S/V, C/V, $\delta^{13}C$ values and C/N ratio for bracken are also within the ranges of values for other plant samples, the sediments from both lochs Creran and Etive could also constitute of bracken because besides the deciduous plants this is also found in abundant in the catchment surrounding the lochs.

4.3.4.2 $\delta^{13}C$ values

The $\delta^{13}C$ values of the plant samples analysed in this study are compared to values from previous studies. The spruce needles gave $\delta^{13}C$ value of –30.55‰, close to the $\delta^{13}C$ value of –30.0‰ reported for *Pinus rigida* needles by Wilson *et al.* (1985). Both *Pinus c.* and *Pinus t.* have $\delta^{13}C$ values of –26.7‰ and –26.4‰ (Hedges and Parker, 1976). Wilson *et al.* (1985) reported the $\delta^{13}C$ values of –29.5‰ for *Pinus rigida* branch. Omitting the $\delta^{13}C$ value –30.55‰ for the spruce, the $\delta^{13}C$ values for other plants analysed in this study such as the oak, birch and bracken ranged from –21.99‰ to –25.05‰.

Hedges *et al.* (1986) had observed the $\delta^{13}C$ values of individual tree leaves average 2.5 more negative than values for the matching woods (-27.6 ± 1.0‰, n=15). In this study it was also found that leaves have more negative $\delta^{13}C$ values than stems. Birch leaves are 2.02 more negative than stem, bracken leaves are 2.54 more negative than stem (Table 4.8).

4.3.4.3 C/N ratio

The C/N ratio of the bracken stems is 63.36 and bracken leaves is 10.18, the C/N ratio of birch stems is 33.40 and its leaves 12.72 (see results in Table 4.8; also Table 3.15c). According to Bashkin (2002), the content of nutrients in leaf tissues is higher and hence the C/N ratio is smaller. The mass loss from stems was significantly lower than that of terrestrial leaves (Koukoura *et al.*, 2003).

Table 4.8 Lignin parameters from plant samples from past and present studies.

Ref	Plant	% composition (Λ in parentheses)				S/V	C/V	(Ad/Al)v	$\delta^{13}C$
		P	V	S	C				
Hedges and Parker (1976)	*Sargassum*	74%							-15.1
	Pinus c.	5	95					0.17	-26.7
	Pinus t.	8	92					0.11	-26.4
	Acer s.		26	74		2.85		0.15	-27.0
	Ilex o. (wood)		16	84		5.25		0.17	
	(bark)		32	65	3	2.03	0.09	0.12	-26.8
	(leaf)		23	63	10	2.74	0.43	0.19	-27.4
	Pheonix d.	26	33	41		1.24		0.22	-23.4
	Spartina a.	7	28	30	35	1.07	1.25	0.14	-11.3
	Canavalia m.	12	30	36	23	1.20	0.77	0.13	-10.9
	Avicennia g.	11	20	39	30	1.95	1.50	0.15	-20.8
Hedges & Mann (1979a)	Nonvascular		0	0	0				
	Gymno wood		4.2-13mg/100mg OC						
	Nonwoody gymno		(1.9-2.1)	(0-0.12)	(0.8-1.2)	0-0.06	0.38-0.57		
	Angio wood		(2.7-8)	(7.2-18)		1.2-5.2			
	Nonwoody angio		(0.7-3)	(0.7-2.9)	(0.45-3.1)	0.44-2.6	0.36-1.1		
Wilson *et al.* (1985)	*Ascophyllum sp.*	92	1						-15.0
	Pinus rigida (branch)	12	79	1	10	0.01	0.13	0.20	-29.5
	(needles)	10	79		15		0.19	0.13	-30.0
	Quercus ilicifolia (branch)	2	38	57	4	1.5	0.11	0.14	-29.8
	(needle)	23	23	26	29	1.13	1.26	0.25	-26.3
	Spartina alterniflora (stem)	6	32	31	33	0.97	1.03	0.13	-12.5
	(detritus)	8	37	36	20	0.97	0.54	0.10	
	Ammophila breviligulata (stem)	5	17	21	60	1.24	3.53	0.15	-25.4

Table 4.7 continued.

Ref	Plant	% composition				S/V	C/V	(Ad/Al)v	$\delta^{13}C$	Others
		P	V	S	C					
This study	Spruce	65.94%	2.07	10.71	21.29	5.18	10.29	0.36	-30.55	C/N = 24.89
	Oak (leaves)	34.38	4.96	12.14	48.52	2.44	9.77	0.54		
	(stems)	7.77	39.92	28.57	23.74	0.72	0.59	0.17		
	Birch (leaves)	13.32	17.36	20.23	49.09	1.17	2.83	0.12	-29.75	12.72
	(stems)	16.01	27.50	3.58	52.92	0.13	1.92	0.03	-27.73	33.40
	Bracken (leaves)	61.38	13.62	16.56	8.44	1.22	0.62	0.06	-27.35	10.18
	(stems)	21.44	44.26	23.58	10.72	0.53	0.24	0.01	-24.81	63.36

Key:

From Hedges and Parker (1976): *Pinus c.* and *Pinus t.*: conifer; *Acer s.*: silver maple dicotyledon; *Ilex o.*: American holly, dicotyledon; *Pheonix d.*: fig palm, monocotyledon; *Spartina a.*: cord grass, monocotyledon; *Canavalia m.*: jackbean, dicotyledon; *Avicennia g.*: black mangrove, dicotyledon

From Wilson *et al.* (1985): *Pinuis rigida*: gymnosperm; *Quercus ilicifolia*: angiosperm; *Spartina alterniflora* (C4, angiosperm)

All P, V, S and C contents are reported as % composition, except that of Hedges and Mann (1979b) reported in mg/100mg.

4.3.5 Summary

1. The S/V and C/V ratios for sediments in both lochs Creran and Etive indicate the presence of non-woody angiosperms tissues, most probably originating from deciduous plants such as birch, oak and beech surrounding the lochs.

2. The decrease of S/V values in both lochs Creran and Etive is due to enhanced degradation of syringyl phenols compared to vanillyl phenols, indicating that syringyl phenols are more susceptible to diagenesis.

3. Overall the order of stability of these phenol groups increase in this order: S≈C < V (Hedges *et al.*, 1985; Hedges *et al.*, 1986; Ertel *et al.*, 1986; Lobbes *et al.*, 2000; Dittmar and Lara, 2001), most probably due to the more easily breakable methyl group and the double bonds in the S and C groups, respectively.

   In Loch Etive, the percentage decrease of phenol groups decreases in this order: C>S>V, indicating that diagenesis of the cinnamyl and syringyl phenols occurred faster than the vanillyl phenols. However, in Loch Creran, the percentage decrease of the phenol groups occur in this order: S>V>C, indicating that the lignin material in Loch Creran is already more degraded, and the hydrodynamic sorting process plays an important role in transporting the terrestrial materials further down the loch, due to entrainment of materials by the incoming denser saline water.

4. Analyses of some known plant samples give several interesting results:
   - The $\delta^{13}C$ values for plant samples are within the $\delta^{13}C$ values reported previously.
   - The $\delta^{13}C$ value for spruce is almost the same as the $\delta^{13}C$ value for *P.rigida* analysed by Wilson *et al.* (1985).
   - The $\delta^{13}C$ values for leaves are more negative than wood (this study and Hedges *et al.*, 1986).

- The C/N ratios of stems are higher than leaves (this study and Bashkin, 2002).

4.3.6 Strengths and weaknesses of past and present studies

1. Vegetation source in both lochs Creran and Etive is determined from the S/V and C/V ratios, and from the lignin compositional plots. The strength of this work is that determination of the vegetation sources in this way was further confirmed using the aerial photographs and via personal observation and by referring to the vegetation types shown in the Ordnance Survey vegetation map.

2. The weakness lies mainly in the lack of plant samples analysed using the CuO oxidation process. Compared with Hedges and Parker (1976), Hedges and Mann (1979a) and Wilson *et al.* (1985), in this study only four plant types were analysed for lignin phenols. Hence it was not possible to construct the lignin phenols compositional plots from these results. Moreover lignin analysis of bark samples was not carried out.

   The spruce samples (Table 4.7) should not have yielded syringyl phenols. More analysis of this sample is needed.

4.4 PROXIES FOR THE BIODEGRADABILITY OF SEDIMENT ORGANIC MATTER

Biodegradation* can be defined as the biologically catalysed reduction in the complexity of chemicals. In the case of organic compounds, biodegradation frequently leads to conversion of the C, N, P, S, and other elements in the original biochemicals to inorganic products. This is also known as mineralization. Ultimately biodegradation is a term sometimes used as a synonym for mineralization. Plant and animal respiration are mineralization processes that destroy numerous organic molecules of living organisms (Alexander, 1999).

According to these authors: Kristensen and Blackburn (1987), Henrichs (1992), Canfield (1994), Kristensen *et al.* (1995), Aller and Aller (1998), Kristensen and Holmer (2001), mineralization rate is determined by the quality of organic matter (Section 1.2.3.1). The quality of organic matter or its biodegradability is the ability of the organic matter to be mineralised or decomposed, hence the lability of the organic matter. Higher biodegradability of sediment organic matter means that the organic matter is more labile and susceptible to organic matter degradation.

According to the Collins English Dictionary, 'proxy' means authorized agent or substitute. Hence the proxies for biodegradability of sediment organic matter are the means or substitutes used to determine the biodegradability or lability of the sediment organic matter. Here the biodegradability of sediment organic matter is measured using these proxies: oxygen uptake rate, percentage organic matter due to loss on ignition (LOI), Rp values, and TC, TN, P and the C/N/P ratios. Stable carbon isotopic ratios are used to determine the relative abundances between marine and terrestrial organic matter, and also discussed in this chapter.

The best proxy should be able to indicate the presence of the more labile+ or refractory fraction of the organic matter. In the case of the lochs, the biodegradability of sediment organic matter

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* **biodegradable** (of sewage constituents, packaging material, etc.) capable of being decomposed by bacteria or other biological means.

+ **labile** (of a compound) prone to chemical change (Collins English Dictionary).

seems to be due to the input of terrestrial organic matter. In order to determine which of these proxies serve best to indicate the biodegradability of sediment organic matter, several studies have been carried out with each proxy:

1. Seasonal and spatial variations along transects of the lochs for each proxy.
2. The proxies are correlated with total lignin, which is the representative of terrestrial organic matter, and correlated among themselves.

4.4.1 Stable carbon isotopes compositions

The $\delta^{13}C$ values of some known plant samples analysed (Section 3.5.1) ranged from -24.67‰ to -30.72‰. The algae samples have $\delta^{13}C$ values from -16.78 to -22.19‰ (Table 3.14a). The $\delta^{13}C$ values of the sediment samples obtained from lochs Creran and Etive ranged from -11.25‰ to -25.76. Omitting the $\delta^{13}C$ values from LC5 and Camas Nathais (as both are situated outside the lochs) the $\delta^{13}C$ values in lochs Creran and Etive ranged from -16.10‰ to -27.76‰ (Table 3.14, c and d; see also Figure 4.10). These $\delta^{13}C$ values are within the values reported from past studies (Tables 4.8 and 4.9).

4.4.1.1 Known compounds

The carbon isotope analyses carried out for several known plant samples gave the $\delta^{13}C$ values of approximately those reported from previous studies. The stable carbon isotopes analyses of several plants such as bracken and birch stems and leaves, and spruce needles (Table 3.14a) gave a mean $\delta^{13}C$ value of –28.04‰. This value is used here to represent the $\delta^{13}C$ value for the C3 terrestrial plant materials, or as the $\delta^{13}C$ – end member for terrestrial organic matter from lochs Creran and Etive. This value is also used in the calculation of the marine and terrestrial fraction of the organic matter (see Section 4.5.2). The $\delta^{13}C$ values of known compounds obtained from this and past studies are given in Table 4.9.

Table 4.9 $\delta^{13}C$ values from past and present studies.

Author (year)	Samples	$\delta^{13}C$ values
Smith and Epstein (1971)	Algae	–12 to –23‰
Hedges and Parker (1976)	Marine alga *Sargassum*	–15
Gearing *et al.* (1984)	Phytoplankton	–23.4 to –19.3
Cerling *et al.* (1993)	Trees, shrubs, herbs and cool-season grasses and sedges using the C3 pathway	–23 to –35
Goni and Hedges (1995)	Brown alga	–14.4 to –22.6
	Green alga	–17.6
	Red alga	–33.1
Trumbore and Druffel (1995)	Plants using C3 photosynthetic pathway	–22 to –32
Goni and Thomas (2000)	C3 plants	–24.5 to –25.8
	Pine forest	–27 to –29
Van Bergen and Poole (2002)	Oak wood	–25.6 to –28.0
This study	Green alga *Tetraselmis chui* and brown alga *Pavlova lutheri*	–17.17 to –22.08
	Vanillin, syringaldehyde and syringic acid standard (Sigma Aldrich)	–27.33, –27.47, and –32.59
	Spruce needles	–30.55
	Bracken leaves and stems	-27.75 and -24.81
	Birch leaves and stems	-29.75 and -27.73

Based on the results in Table 4.8, a scale for the $\delta^{13}C$ values is constructed (Figure 4.9). This scale indicates that the $\delta^{13}C$ values for marine algae range from –12‰ to –23‰, and the $\delta^{13}C$ values for terrestrial materials range from –24.81‰ to –32.59‰. The $\delta^{13}C$ values obtained from sediment samples can then be compared to this scale in order to determine the relative abundances between the terrestrial and marine organic matter in the sediments.

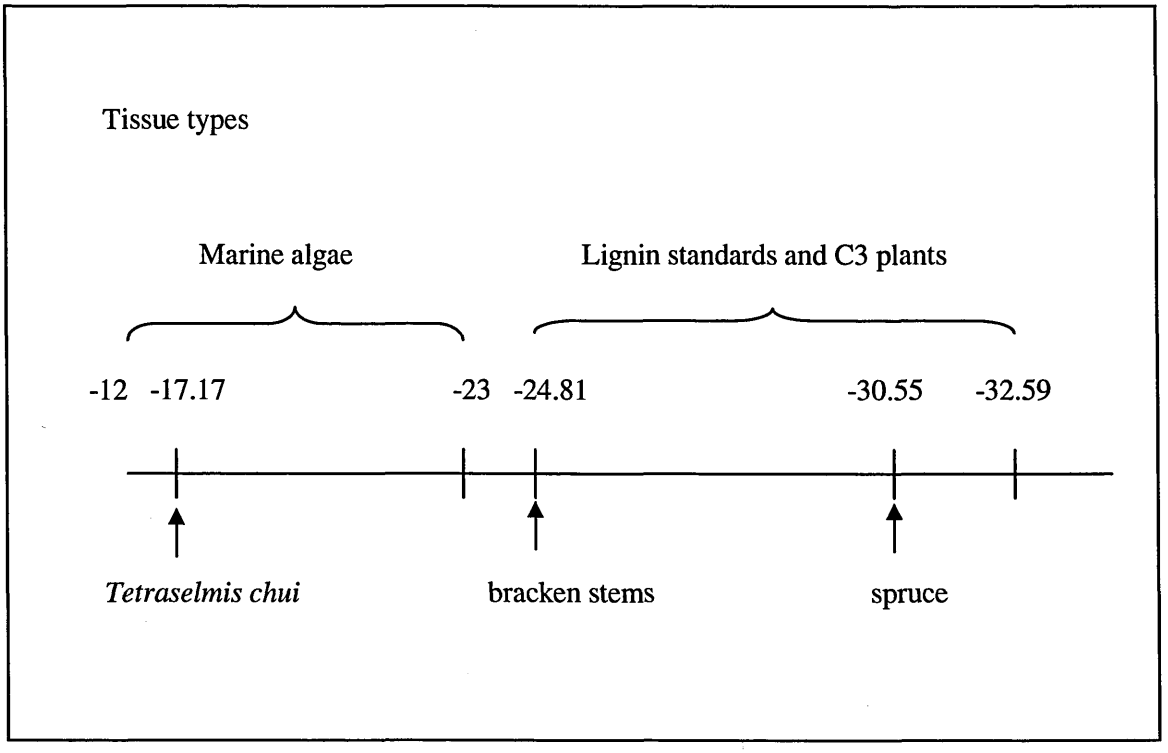


Figure 4.9 $\delta^{13}C$ values for known compounds. The ranges of $\delta^{13}C$ values for different plant samples are based on results from this study, and results from previous studies: Smith and Epstein (1971), Hedges and Parker (1976), Gearing *et al.* (1984), Cerling *et al.* (1993), Goni and Hedges (1995) and Goni and Thomas (2000). The labelled $\delta^{13}C$ values for the known compounds analysed in this study are *Tetraselmis chui*, bracken stem and spruce. See text for details.

Implications

1. Compilation of past and present studies gives ranges of the $\delta^{13}C$ values of marine algae from -12 to -23‰, and for terrestrial materials from -24.81 to -32.59‰.
2. The $\delta^{13}C$ values for our analyses of known plant samples were within the range of values reported from previous studies (see references in Tables 4.8 and 4.9).
3. The $\delta^{13}C$ values in lochs Creran and Etive ranged from -16.10‰ to -27.76‰.
4. This serves as confirmation of the validity of the carbon isotope analysis.
5. Also, this means that the $\delta^{13}C$ values can be used to determine the relative abundance of the terrestrial versus marine organic matter.

4.4.1.2 Seasonal variations

The $\delta^{13}C$ values of sediments from lochs Creran and Etive are within the ranges found in other locations (Table 4.2).

Implications

1. Sediment trap samples showed elevated (more enriched) $\delta^{13}C$ values to –19‰ from April to June 2002, compared to the $\delta^{13}C$ values from other months. From December 2001 to March 2002, the $\delta^{13}C$ values ranged from –24.66 to –22.48‰, from April to June 2002 the $\delta^{13}C$ values ranged from –19.65 to –19.80‰, and from July to December 2002 ranged from –21.21 to –21.37‰. The slight increase from April to June 2002 indicates the contribution of marine organic matter.

   Eadie and Jeffrey (1973), Mitchell *et al.* (1997) and Lehmann *et al.* (2004) also found similar conditions: increase of $\delta^{13}C$ values indicating increase in the phytoplankton detritus during summer, and decrease of the $\delta^{13}C$ values in winter.

2. At LC1, the isotopic composition recorded for the three months (June, September and December 2002; see Table 3.14c) shows constancy of –23.13‰, -23.20‰ and –23.98‰ for the 0-1cm sediment layer, and –24.33‰ and –24.57‰ for the 9-10cm layer. Overall the mean $\delta^{13}C$ value for the sediment trap sample was higher (mean=-21.33±0.23‰) with a more elevated value of –19.80‰ in June 2002, whilst the $\delta^{13}C$ values for sediment at LC1 was constant with time. This means that the sediment trap has collected more phytoplankton material. According to these results in the uppermost basin of Loch Creran, the phytoplankton material was mostly degraded in the water column, resulting in minimum phytoplankton detritus settling onto the surface sediments. Hence there was minimal change in the $\delta^{13}C$ values in the surface sediments at LC1.

3. At individual locations, only the lignin concentration and $\delta^{13}C$ values from the sediment trap sample are significantly correlated (simple regression analysis: r=-0.68, $p<0.05$; Section 3.5.3). This is most probably because the sediment trap is situated in the water column and is the first to receive organic matter input from River Creran. This good correlation indicates that both $\delta^{13}C$ values and lignin material are sensitive to detect the abundance of terrestrial and marine materials.

4.4.1.3 Transects of the lochs

In Loch Creran, $\delta^{13}C$ values for sediment trap samples ranged from –24.96 to –19.29‰ (Table 3.14b), the $\delta^{13}C$ values for LC0 and LC1 ranged from –25.21 to –23.13‰, LC2 and LC3 ranged from –24.03 to –16.10‰, LC5 ranged from –20.96 to –17.14‰, and LC6 ranged from –15.18 to –14.30‰. Figure 4.10 illustrates these ranges more clearly.

From Figure 4.10, RE2, RE5 and RE6 all have the most negative $\delta^{13}C$ values while Camas Nathais has the most positive $\delta^{13}C$ value. Spanning this range are the $\delta^{13}C$ values from locations along Loch Creran. The most negative $\delta^{13}C$ values in the Creran head, LC0 and LC1 at the upper most basin in Loch Creran indicate the presence of terrestrial organic matter. The $\delta^{13}C$ values of sediment trap samples span a wide range. Comparison with Figure 4.9 shows that most of the materials collected in the traps are of marine origin. The $\delta^{13}C$ values of both LC2 and LC3 in the middle basin of Loch Creran spanned the widest range, as LC2 was still influenced by terrestrial organic matter whereas LC3 was influenced by marine organic matter.

Due to its location, the $\delta^{13}C$ values for LC5 should range between the $\delta^{13}C$ values between LC3 and LC6. In reality, these higher $\delta^{13}C$ values obtained for LC5 could be because LC5 is quite sheltered due to its location between two bends, allowing terrestrial materials from the upper loch to accumulate here. The $\delta^{13}C$ values for LC6 indicate the presence of marine organic matter.

Overall the $\delta^{13}C$ values increase from the head to the mouth and outside the lochs, as found by other authors (Table 4.10).

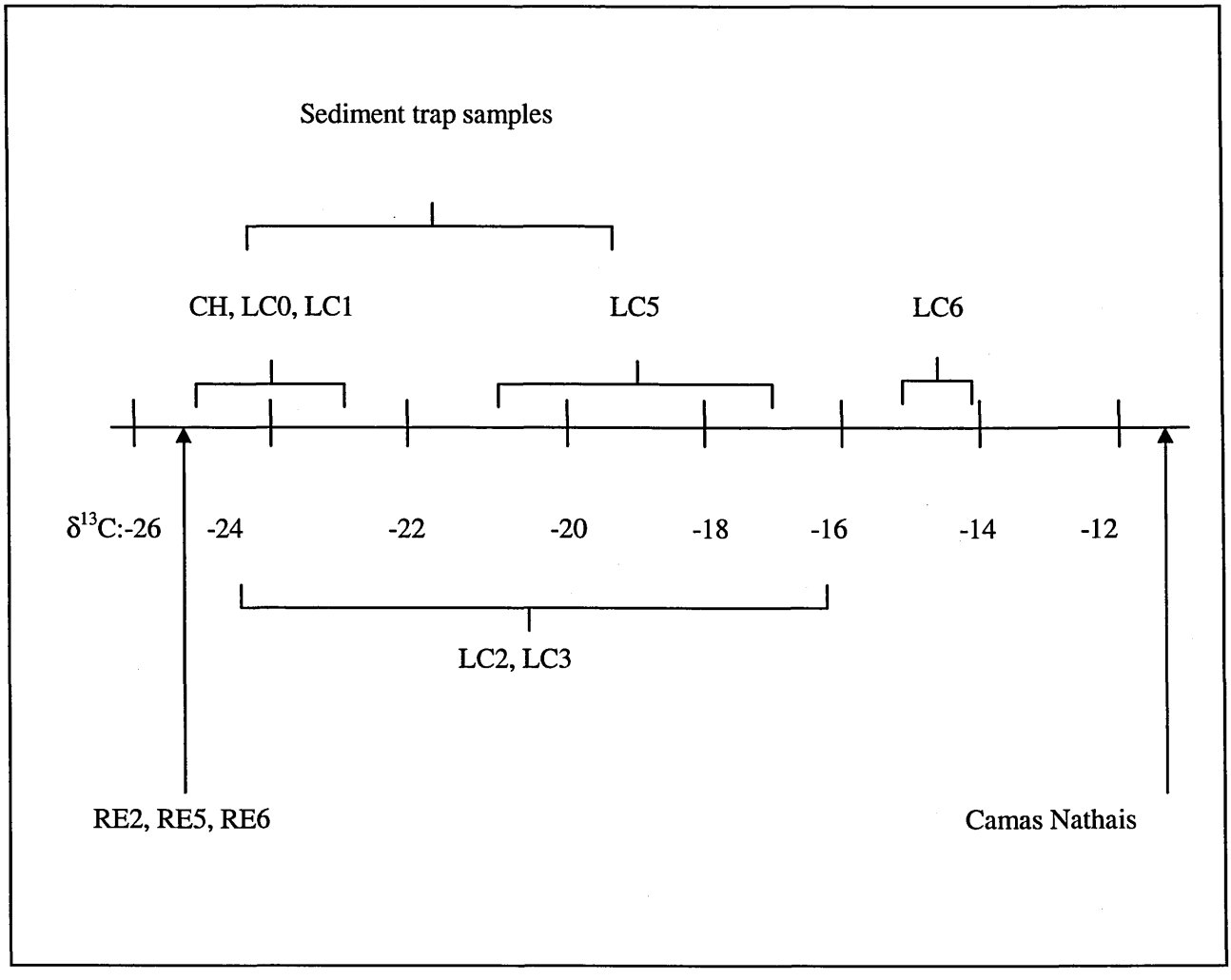


Figure 4.10 $\delta^{13}C$ values for sediments from Loch Creran and Loch Etive. "CH" means Camas Nathais.

"CH" means Camas Nathais.

Table 4.10 Coastal and offshore decreased in $\delta^{13}C$ values.

Author (year)	$\delta^{13}C$ values increase offshore, previous results.
Degens (1969)	The mean $\delta^{13}C$ of freshwater sediments is –25‰, and for marine sediments –20‰.
Hedges and Parker (1976)	Offshore decrease in lignin concentrations and increase carbon isotope ratios.
Gearing *et al.* (1977)	The Mississippi, Niger and Atchafalaya rivers have $\delta^{13}C$ values around –24‰, and 10km offshore increased to around –20‰.
Tan and Strain (1979)	Isotopic composition increased with increase distance from river source.
Gearing *et al.* (1984)	Upper Providence River and nearshore stations have more negative isotope ratios (-22.0 to –24.7‰), and further offshore $\delta^{13}C$ values increased (-20.6 to –23.6‰).
Fichez *et al.* (1993)	Increased of the $\delta^{13}C$ values from the river (-30‰) to the tidal freshwater (-22‰).
Ruttenberg and Goni (1997)	Increased of $\delta^{13}C$ values with increase distance offshore for sediments from the Arctic.
Cai *et al.* (1988)	The $\delta^{13}C$ values for rivers in the Peruvian Amazon, the upper reach of Amazon, lie between –24.5 and –28.0‰. More negative $\delta^{13}C$ values are observed for the middle and lower reaches of the Amazon main channel –27.9 to –30.1‰.
Yamamuro (2000)	Found strong negative correlation with the distance from the seawater entrance.
Goni *et al.* (2000)	Increased in the $\delta^{13}C$ values further offshore from the Mackenzie River to Beaufort shelf suspended river sediments: -26.5 to –25.4‰.
Zhu *et al.* (2002)	Increased in the $\delta^{13}C$ values from between –26‰ and –25.9‰ inshore, to between –14‰ and –18.6‰ offshore.
Gordon and Goni (2003)	Depleted stable carbon isotopes values of typical C3 plant debris (-27‰) are found near the river mouth and become more enriched (-22 to –21‰) offshore.

Implications

1. There is significant correlation (simple regression analysis: $p<0.05$) between total lignin and $\delta^{13}C$ values for Loch Creran ($r=-0.76$) and Loch Etive ($r=-0.96$) transects (Section 3.5.3), indicating that nearest the river input there was the highest amount of lignin material and most negative $\delta^{13}C$ values. Further down the loch, total lignin decrease and the $\delta^{13}C$ values increased.

Besides lignin, the only other proxy which the $\delta^{13}C$ values have significant correlation with was the Rp values (regression analysis: $r=0.86$, $p<0.05$; Section 3.8, Table 3.21b). As the $\delta^{13}C$ values are indicator of the relative abundance between terrestrial and marine organic matter (hence the significant correlation of $\delta^{13}C$ values with lignin content), these results also indicate that the Rp index is another powerful tool to measure the lability of the sediment organic matter (Rp values also correlates significantly with total lignin; Section 3.8, Table 3.21a).

2. The 9-10cm sediment layers have more negative $\delta^{13}C$ values than the surface 0-1cm layer (Table 3.14c) for all the sampling locations in Loch Creran, indicating that some fresher marine organic matter might have settled on the surface sediments, except for the Loch Creran head where surface sediment was found to be more depleted ($\delta^{13}C$ = -25.04‰) than the 9-10cm layer ($\delta^{13}C$ value = -23.86‰). This could be due to very rapid sedimentation rate at that time, resulting in fresher materials being deposited without having undergone degradation.

4.4.2 Oxygen uptake rate

The oxygen uptake rates were measured only in Loch Creran, and ranged from 6.63 to 27.78 mmoles/m^2/day (Section 3.3.1; Tables 3.7). Parkes and Buckingham (1986) found that the oxygen uptake rates in Loch Etive ranged from 13.6 to 17.8 mmole/m^2/day, in Loch Eil ranged 17.8-24.6 mmole/m^2/day and in Tay Estuary ranged 67.4 mmole/m^2/day. Overnell *et al.* (1995b) found that the oxygen uptake rates in Lochs Linnhe, Goil, Fyne and Etive ranged from 8 to 24 mmole/m^2/day at ambient temperature (9-11°C). Hence the oxygen uptake rates in Loch Creran are mostly within the values reported for other locations on the west coast of Scotland. Accornero *et al.* (2003) found that the oxygen uptake rates in Gulf of Lions continental margin ranged from 0.6 to 48 mmole/m^2/day.

4.4.2.1 Seasonal variation

Oxygen uptake rate at LC1 increased significantly from June (18.67 mmoles/m^2/day) to July (26.36 mmoles/m^2/day), peaked in August (27.78 mmoles/m^2/day) and decreased significantly from September (23.52 mmoles/m^2/day) to October (18.87 mmoles/m^2/day) 2002 (Section 3.3.1). Oxygen uptake rate increased slightly during the summer due to increase in the oxygen consumption as a result of the increase in microbial activity in the summer (Tett and Wallis, 1978; Wassman, 1984; Parkes and Buckingham, 1986; Grant and Hargrave, 1987; Overnell *et al.*, 1995b; Papadimitrou *et al.*, 2002). The highest oxygen uptake rates were recorded in late spring (May), when values were 1.5 to 4.2 times higher than in other seasons (Accornero *et al.*, 2003).

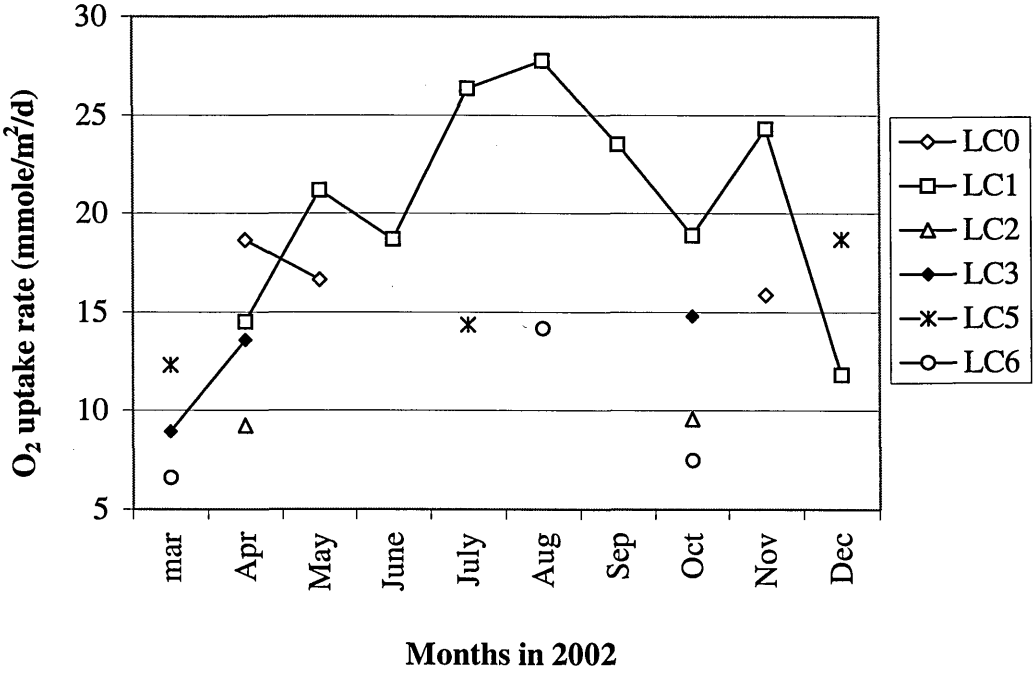


Figure 4.11 Seasonal variations in the oxygen uptake rates at individual locations.

From Figure 4.11, it is seen that at LC0, the oxygen uptake rate decreased from April to May 2002. The oxygen uptake rates at LC1 throughout the year show the most significant increase during the summer months. At LC2, the oxygen uptake rates between the two months April and October did not differ much. At LC3, the oxygen uptake rate increased from March to April, but the rate in April did not differ much from the rate in October. At LC5, the oxygen uptake rate increased towards the end of the year. LC6 is the other location showing prominent increased in the oxygen uptake rate during the summer, most probably due to the input of marine organic matter, as LC6 was situated outside the loch, and therefore received the least input of terrestrial materials.

Implications

1 The increase of the oxygen uptake rate during the summer indicates the input of marine organic matter as this fresh organic matter is more susceptible to degradation. The increase of oxygen uptake rates during the summer is also evidenced by the significant correlation (simple regression analysis: $r=0.76$, $p<0.05$) between the oxygen uptake rate and temperature (Section 3.3.2; Table 3.8).

4.4.2.2 Transects of the lochs

There is however, significant decrease of the oxygen uptake rate from the head to the mouth of Loch Creran. The highest oxygen uptake rate occurred at LC1 (20.78 mmoles/m^2/day) and decreased to 9.44 mmoles/m^2/day at LC6. At LC5 however, the oxygen uptake rate showed a slight elevation. Rowe *et al.* (1994), Overnell *et al.* (1995b) and Accornero *et al.* (2003) had also found that the oxygen uptake rate increased near the heads of lochs, implying the presence of a component of terrestrial input readily degradable by marine sediment bacteria.

Implications

1. Three factors influence the sediment oxygen uptake rate: temperature, the amount of organic material falling onto the surface sediment and the flow rate of water over the surface sediment (Davies, 1975; Smith, 1978).

   In this research, the effect of temperature was cancelled because the oxygen uptake rate was measured at a constant temperature of 10°C (Section 2.2.4).

   As the oxygen uptake rate decreased significantly from the head to the mouth of Loch Creran, the most probable reason would be due to the contribution of terrestrial organic matter. Locations nearest the River Creran received the highest input of fresher terrestrial materials and these locations have the highest oxygen uptake rate. Hence the amount of organic matter falling onto the surface sediments plays an important role in affecting the oxygen uptake rate in Loch Creran. The rate of community respiration was related to the input of fresh organic matter. This also implies that the benthic community metabolism was regulated by the food supply (Davies, 1975).

2. As the degradation of fresh organic matter proceeds regardless of the oxygen availability (Section 1.2.3) and that the oxygen uptake rates along Loch Creran decreased from the head to the mouth and outside the loch (Sections 3.3, Figure 3.8), another implication is that the oxygen uptake rates in Loch Creran were due to the effect of 'old' materials in terms of the relativity between terrestrial and marine organic matter (as terrestrial organic matter is more refractory and older than marine organic matter). These terrestrial materials at the head of the loch, however, were considered fresher than the terrestrial materials further down the loch, as further down the loch, these materials would be more degraded. Hence the oxygen uptake rates along Loch Creran were due to the degradation of the 'older' but still degradable terrestrial organic matter. According to Wollast (1991),

the organic matter freshly deposited at the sediment-water interface undergoes rapid degradation by aerobic bacterial respiration.

3. The oxygen uptake rate was found to be highest at locations nearest River Creran and the rates decreased further down the loch. This is most probably due to the contribution of terrestrial organic matter. This is supported by two other conditions: the most negative $\delta^{13}C$ values near the river input, and the highest lignin content. Also this means there was an input of fresher terrestrial material at the head of the loch, as the decomposition rate of the nitrogen-rich litter was initially higher than older materials (Berg *et al.*, 1982).

4. Further down the loch, the oxygen uptake rate decreased significantly, as the contribution of terrestrial organic matter decreased, and the terrestrial organic matter has already been degraded.

5. Towards the mouth of the loch, marine organic matter predominates. Hence the overall decrease of oxygen uptake rate down the loch indicates that the overall effect on the sediment biodegradability is governed by the terrestrial organic matter input into the loch.

4.4.2.3 Other factors

Parkes and Buckingham (1986) found that the highest oxygen uptake rate occurred at the shallowest site and the oxygen uptake rate decreased with increase depth of the overlying water. There is however, no significant (ANOVA: $p>0.05$) difference between the oxygen uptake rates in Loch Creran with the locations' depths. Hence the depths of the overlying water do not affect the biodegradability of the sediment organic matter. LC1 is the deeper station compared to LC0, yet has the higher oxygen uptake rate (Table 3.7, Section 3.3.1).

The oxygen uptake rate does not correlate significantly with any other proxy except with the inorganic phosphate content (simple regression analysis: $r=0.98$, $p<0.05$) for the Loch Creran transect (see Section 4.4.5 for details).

As sedimentary organic matter consists largely of material resistant to decomposition, there is only poor correlation between this material and the degradation activity rates such as the oxygen consumption and sulphate reduction (Overnell *et al.*, 1995b), hence no significant correlation was observed between the oxygen uptake rates and other parameters.

Lignin concentration decreased offshore (Figures 3.5 and 3.6a; Section 3.2.1.3), so was the oxygen uptake rate (Figure 3.8; Section 3.3.1). Hence the question arising would be why there was no correlation between the lignin content and oxygen uptake rates. Comparison between Figures 3.6a and 3.8 shows that, the lignin content decreased, but oxygen uptake rate increased from LC0 to LC1. The reason for the decreased in lignin concentration at LC1 is due to transportation and the terrestrial materials sedimented at location nearer to the river input, that is, LC0. The reason for the increase in the oxygen uptake rate at LC1 could be because LC1 is situated nearer the narrows at Creagan, hence the effect of entrainment is stronger here, resulting in better water circulation.

Among the four locations situated further down the loch, lignin content decreased from LC2 to LC6 (Figure 3.6a), with the exception of slight increase at LC5, which was most probably due to accumulation of lignin material here as this location is quite sheltered. The decrease of lignin concentration down the loch is due to transportation and accumulation. The oxygen uptake rates were higher at LC3 and LC5 (Figure 3.8), most probably due to local topographic features. LC3 is a deeper basin (49m; see Table 4.21, Section 4.6.2.2), resulting in accumulation of more materials and LC5 is a more sheltered location.

The distribution of lignin along the loch is due to its transportation and accumulation along the loch. The fluctuation in the oxygen uptake rates was also affected by the topographic features of

the individual locations. Overall both parameters decreased significantly from the head to mouth of the loch, indicating the contribution of terrestrial organic matter from River Creran.

4.4.3 Loss on ignition

Loss on ignition (LOI) parameter ranges in Loch Creran is given in Table 4.10. The wide ranges of % labile organic matter, % refractory organic matter, % total organic matter and the Rp values are observed for sediments from the sediment trap, LC0, LC1 and LC5. The most probable reason is the input of new organic matter into these locations. LC0, LC1 and the sediment trap were most probably affected by the input of terrestrial organic matter from the River Creran. LC5 is a sheltered basin accumulating materials from the upper loch. Further down the loch, LC2 in the next basin and LC6 outside the loch, the organic matter contents become more constant. For Loch Etive, percentage organic matter was extremely consistent (see Table 3.11h). The larger range observed in Table 4.11 is due to the increase in the percentage organic matter fractions at RE6 for March 2001.

Table 4.11 Ranges of the loss on ignition parameters.

Location	% labile	% refractory	% total organic matter	Rp
Sediment trap	9.31-20.90	5.77-11.18	15.58-28.84	0.28-0.45
LC0	8.78-10.25	4.83-8.76	13.61-18.71	0.36-0.47
LC1	2.96-12.70	0.69-8.84	3.65-20.54	0.19-0.57
LC2	5.99-7.94	5.69-6.46	12.24-13.63	0.42-0.51
LC3	2.76-4.77	3.78-6.02	6.36-10.79	0.53-0.63
LC5	0.86-12.11	1.39-7.64	2.25-19.74	0.39-0.67
LC6	3.28-4.05	4.96-6.91	8.24-10.96	0.60-0.68
RE2, RE5, RE6	10.88-12.77	6.75-18.73	17.69-29.62	0.34-0.46
Camas Nathais	3.04	12.08	15.11	0.75

The % total organic matter in Loch Creran due to loss on ignition ranged from 2.25 to 28.84%. In Loch Etive, the % total organic matter ranged from 15.11 to 29.62% (Section 3.4.3 and Table 4.12). Parkes and Buckingham (1986) found that the % total organic matter due to loss on ignition for Loch Etive ranged 17-19%, in Loch Eil ranged 14-15% and in Tay Estuary 7%. Overnell *et al.* (1995b) found that the % total organic matter in Lochs Linnhe, Goil, Fyne and Etive ranged 3-20%. Hence the % total organic matter found in this study for lochs Creran and Etive are within or slightly higher than the values reported for other locations on the west coast of Scotland (Table 4.12).

Table 4.12 Loss on ignition results from previous studies.

Author (year)	Findings
Parkes and Buckingham (1986)	Reported the % total organic matter content (due to LOI) for Loch Etive ranged 17-19%, Loch Eil ranged 14-15%, and Tay Estuary 7% organic matter. The three locations in Loch Etive in Parkes and Buckingham (1986) studies were located between RE2 and RE5 in this study.
Overnell *et al.* (1995b)	Found that the highest organic matter concentrations in the top 2cm (as measured by LOI) tend to occur at the deepest sites. Organic matter concentrations also showed elevation at the heads of Lochs Fyne and Etive, possibly reflecting the input of more recalcitrant organic fractions of terrestrial origin. These elevated values reflect the degradation of riverine particulate organic carbon (POC) input, most of which are expected to have settled near the heads of the lochs. The % organic matter obtained from the histograms for Loch Goil ranged 7.5-20%, Loch Fyne 2.5-15%, Loch Etive 11-17.5% and Loch Linnhe 2.5-10%. These three locations in Loch Etive were equivalent to RE2, RE5 and RE6 in this study.

4.4.3.1 Seasonal variations

Overall there was only slight seasonal variation in the LOI parameters at individual locations. For the sediment trap samples, there were slight increases in both % labile and % refractory

organic matter, but decrease in the Rp values from June to September 2002. Overall the sediment trap samples showed more variation for the % labile organic matter. At LC0, there was extreme constancy of these LOI parameters during the duration studied. At LC1, there were some increases and decreases but these were of no seasonal significance. At LC5, there was a huge increase in the percentage organic matter parameter in July 2002, especially the labile fraction, as also indicated by the low Rp index.

4.4.3.2 Transects of the lochs

Lignin serves as the biomarker for terrestrial organic matter. The decrease of lignin from the head to the mouth and outside the lochs signifies the decrease of the terrestrial organic matter. The decrease of % labile organic matter down the loch, and the significant correlation between the % labile organic matter with total lignin indicate that the labile fraction of the organic matter is closely associated with lignin. Overall there are significant decreases of percentage organic matter parameters. The increase of the Rp values from the head to the mouth of the lochs indicates the presence of more refractory organic matter further down the lochs.

Implications

1. The percentage organic matter due to loss on ignition consisted of TC, TN and lignin, as indicated by the significant correlations between these parameters. Table 3.21b shows that % labile and % total organic matter have significant correlation (simple regression analysis: $p<0.05$) with %TC and %TN, indicating that the % labile and hence % total organic matter constitute of %TC and %TN.

2. At individual locations, there was continual and intermittent input of materials throughout the year, especially at LC1 (Table 3.11c). Intermittent input of materials most probably occurred in circumstances when there was a high rainfall event. The

explanation for this phenomenon is the same as the explanation for the lignin content (Section 4.3.2.1: Implication 1). According to Weintraub and Schimel (2003), the lack of changes in the organic matter quality indicates that this material had already decomposed to the point where the breakdown of the labile constituents was tied to lignin decomposition. This could be the reason for the significant correlation between lignin and % labile organic matter.

3. As the percentage organic matter due to loss on ignition (LOI) have significant (ANOVA: $p<0.05$) correlation with the lignin contents (Section 3.8, Table 3.21a), and the total lignin was affected slightly by the seasonal variation (Section 4.2.2.1, Implication 2; Section 4.3.1.1, Implication 3), the effect of rainfall and sedimentation rate on the percentage organic matter measured by LOI is also investigated here.

   The increased of rainfall during February and June 2002 (Section 3.1.1, Figure 3.1c) result in increased in sedimentation rates in April and August 2002 (Section 3.1.2, Figure 3.3) also result in significant increased (ANOVA: $p<0.05$) in the % labile and % total organic matter in the sediment trap samples during February, March and June 2002 (Section 3.4.3, Table 3.11a) and significantly increased in the % labile and % total organic matter at LC1 during March, June and August 2002 (Section 3.4.3, Table 3.11c).

4. The decrease of organic matter content from the head to the mouth and outside the lochs is more significant, hence the contribution of terrestrial organic matter to the bulk organic matter along the loch is more significant compared to the effect of the terrestrial organic matter at individual locations.

5. Increase of the Rp index from the head to the mouth and outside the lochs indicates the increase in the refractory fraction to the total organic matter, as the more labile fraction is degraded during transport.

4.4.4 CN analysis

4.4.4.1 Seasonal variation

Overall the %TC, %TN and C/N ratios are constant at individual locations, as the total sedimentary organic matter is quite constant at individual locations. For the sediment trap samples (see Table 3.17a), the %TC and %TN was slightly higher from May to August 2002 (%TC=7.16-7.90; %TN=0.96-1.07), but the C/N ratios were slightly lower (C/N=7.14-7.46). This could be due to the input of marine organic matter because of the slightly lower C/N ratios. Some authors also found increased input of carbon and nitrogen during winter when the river flow was high, and reduced input of materials when the flow was minimal (Table 4.13).

At LC1 (see Table 3.17b) apart from the huge decrease of %TC, %TN and C/N ratio in February 2002, these parameters are extremely constant throughout the year. Ishiwatari and Uzaki (1987) and Mitchell *et al.* (1997) also found constant carbon content at individual locations.

At LC0, LC2 and LC3, there were decreases in %TC and %TN over the year. This could be due to the input of terrestrial organic matter from River Creran into the loch at the beginning of the year has been gradually transported further down the loch towards the mouth with time. At LC5, there were huge increases of both %TC and %TN in July 2002. At LC6, %TC and %TN decreased from March to August 2002.

Table 4.13 Seasonal variation of %TC and %TN from previous studies.

Author (year)	Findings
Naiman and Sedell (1979)	The highest concentrations of particulate organic detritus were in the winter and lowest concentrations during autumn when flow is minimal
Ishiwatari and Uzaki (1987)	Found that except for the surface sediment above 20m with a high TOC content of 10.5 mgC/g, the carbon content of sediments above 250m (4.4-7.9 mgC/g sediment) exhibits no clear trend with increasing depth.
Cauwet and Mackenzie (1993)	Found that during the winter the organic matter in the suspended load of both estuarine systems was dominantly of terrestrial origin, while in the summer, there were significant contributions from aquatic production.
Argyrou *et al.* (1997)	Found maximum phytoplankton production in the summer and peak allochthonous inputs in the spring and winter or late fall. They found the dissolved organic carbon in Lake Pontchartrain estuary was the highest during high river inflow.
Bianchi and Argyrou (1997)	Found that the highest POC and SPM concentrations occurred during winter and early summer, when freshwater discharge was at their maximum.
Mitchell *et al.* (1997)	Found no significant seasonal differences in organic and inorganic carbon contents despite changing productivity in the water column.
Bianchi *et al.* (2002)	The highest C and N values (in the river) occurred during the lowest flow period in November 1999 and February 2000 when the river discharge dropped below <5000 $m^3 s^{-1}$.

Implications

1. The slightly higher TC and TN accompanied by slightly lower C/N ratio input into the sediment trap during the summer was due to increase input of phytoplankton materials during summer. Of all the locations, only the sediment trap samples showed higher input due to marine organic matter in the summer because the sediment trap was located in the water column.

2. There is overall constancy of %TC, %TN and C/N values especially in the sediment trap, LC1, LC2 and LC6 sediment samples, indicating continual and intermittent input of materials, and constant transport of materials further down the lochs. The explanation

for this is the same as the explanation given for the lignin distribution along the lochs (Section 4.2.2.1: Implication 1).

3. The effect of the rainfall and sedimentation rate as on the lignin content, and percentage organic matter due to LOI (Section 4.2.2.1, Implication 2; Section 4.3.1.1, Implication 3; Section 4.4.3, Implication 3) is seen in the significant increased (ANOVA: $p<0.05$) in the %TC in April 2002 for the LC1 sediments (Section 3.6.3, Table 3.17b).

4.4.4.2 Transects of the lochs

Along transects of both lochs, %TC, %TOC and C/N decreases significantly from head to mouth. Also, total lignin shows significant correlation with %TC, %TN and also %TOC along transect of Loch Creran (Section 3.6.3; Table 3.19a, Section 3.6.4), indicating that lignin also contribute to the total carbon. Hence riverine input contributed to a substantial amount of carbon into the lochs. The %TC and %TN also have significant correlations (regression analysis: $p<0.05$) with the % labile and % total organic matter (Table 3.21b, Section 3.8) indicating that %TC and %TN constitute of the % labile and hence % total organic matter.

4.4.4.3 The C/N ratios

The atomic C/N ratio in living biomass ranges from about 7 (marine microbes) to more than 100 for woody terrestrial materials. In dead organic matter, this ratio increases during burial and diagenesis, as a function of nitrogen release and recycling. The C/N ratios <10-15 stimulate mineralization of organic matter with release of mineral nitrogen, whereas C/N >30 decrease mineralization and increase assimilation (Bashkin, 2002; Månsson and Falkengren-Grerup, 2003). The C/N ratios of various samples obtained by previous authors are given in Table 4.14.

Table 4.14 The C/N ratios of various samples by previous authors.

References (year)	Samples	C/N ratios
Goni and Hedges (1995)	Green and red algae	8-12
	Phytoplankton	7
	Zooplankton	5
	Bacteria	4
Hedges *et al.* (1988a)	Sediment traps samples	7.5
Overnell and Young (1995)	Sediment traps samples	6.87-16.38
Schubert and Calvert (2001)	Indicates marine organic matter	4-8
Zimmerman and Canuel (2001)	Algal organic matter	8-10
Kristensen and Hansen (1995)	Labile and nitrogen-rich detritus	4-5
Gordon and Goni (2003)	Terrigenous signal	29
Zimmerman and Canuel (2001)	Terrestrial organic matter	14-22

Along transects of the lochs, the C/N ratios decreased from 9.31 at LC0 to 4.32 at LC6 in Loch Creran, and in Loch Etive decreased from 13.68 at RE2 to 7.57 at Camas Nathais. Bianchi and Argyrou (1997), Bianchi *et al.* (2002), and Chen *et al.* (2003) found similar phenomenon. This is because during the initial stage of organic matter degradation, the C/N ratios increase due to nitrogen utilization and in the later stage of degradation the C/N ratios decrease due to N immobilization (Benner *et al.*, 1991). Fresher plant materials have higher nitrogen content, and decrease more rapidly (Waksman and Tenney, 1927). The lowering C/N ratios involve the absorption of ammonia derived from decomposition of organic matter accompanied by the remineralization and release of carbon (Meyers, 1997).

4.4.5 Phosphate

Mean values of total phosphate (TP) and total inorganic phosphate (IP) were the highest at LC1 and this is followed by LC5. Apart from LC5, TP and IP decreased from the head to the mouth of the loch. Fang (2004) also found higher phosphate in the inner compared to the outer shelves. High total and inorganic phosphate at LC5 may be emanating from the nearby fish farm. The overall decrease of the mean TP and IP from the head to the mouth of Loch Creran most probably is due to the input of terrestrial materials from the River Creran. This is because the IP has significant (ANOVA: $p<0.05$) correlations with the oxygen uptake rate, % labile and % total organic matter, as well as with %TC and %TN. Organic phosphate (OP) has significant correlation with tbe % labile organic matter, %TC and %TN.

Oxygen uptake rate has significant correlation with only the IP. Redfield (1942) found similar phenomenon, due to organic matter decomposition utilizing oxygen and liberating inorganic phosphate. Upon death and decomposition of organisms and plants a portion of this phosphorus is returned to the water (Grashoff, 1976), hence only small amounts of IP and OP were detected in the sediments.

Marine phytoplankton have a mean molar organic C:P ratio of 106:1, and are relatively impoverished in P and N, with characteristic C:P ratios ranging from 300 to 1300 and C:N ratios ranging from 10 to 100 for soft tissues, C:P ratios greater than 1300 and C:N ratios ranging from 100 to 1000 for woody tissues (Ruttenberg and Goni, 1997). According to Bashkin (2002), the nutrients vary insignificantly, 143-165 for C/N, 1246-1383 for C/P and 8.40-8.80 for N/P for the total plant biomass. Cell detritus losses P preferentially and particulate organic matter reaching the sediment often has a high C:P ratio (Bashkin, 2002).

The calculation of the C/N/P ratios is given in Table 4.15. The C/N/P ratios would be very large due to the small amount of the total phosphate. The C/N and C/P ratios decline as organic matter decomposition proceeds (Bashkin, 2002). The high N/P ratios indicate an overall phosphate

deficiency in the system (Luccea *et al.*, 2003), such that the sediment in Loch Creran is extremely phosphate-deficient.

Table 4.15 Calculation of the C/N/P ratios.

Location	%OC	OC (g)	%TN	TN (g)	Total phosphate (g/g)
Sedi trap					0.744×10^{-6}
LC0	4.77	0.0477	0.52	0.0052	
LC1	4.01	0.0401	0.47	0.0047	0.109×10^{-6}
LC2	3.14	0.0314	0.38	0.0038	0.059×10^{-6}
LC3	1.83	0.0183	0.28	0.0028	0.055×10^{-6}
LC5	2.30	0.0230	0.34	0.0034	0.077×10^{-6}
LC6	1.07	0.0107	0.24	0.0024	0.025×10^{-6}

Sundby *et al.* (1992) found that the top cm sediment in the Laurentian Trough in the Gulf of St. Lawrence has 6±3 mmole PO_4 per liter. The mean total phosphate from all the locations in Loch Creran (Table 4.11) is 0.18 µg/g.

Converting 0.18 µg/g to µmol/L:

0.18/94.97 µmol x $1/(10^{-3}L)$ = 1.90 µmol/L [molecular weight of PO_4 is 94.97]

Hence the sediment in Loch Creran has low phosphate content.

4.4.6 Summary

4.4.6.1 Seasonal variation

- There is overall constancy of all these experimental parameters at individual locations as shown by the mostly no significant difference (ANOVA: $p>0.05$) or inconsistent increase or decrease of these parameters between subsequent sampling months: lignin content (Section 3.2.1.1, Table 3.2), oxygen uptake rate (Section 3.3.1, Table 3.7), percentage

organic matter due to loss on ignition (Section 3.4.3, Table 3.11), $\delta^{13}C$ values (Section 3.5.2, Table 3.14), %TC, %TN and C/N ratio (Section 3.6.3, Table 3.17). All these indicate continual and intermittent input of terrestrial debris into the lochs and constant transport of organic matter out of the lochs.

- The slight increase of rainfall (seasonal variation; Section 3.1.1) resulting in increase in the sedimentation rate (Section 3.1.2), will result in increase in the lignin content, percentage organic matter due to loss on ignition, and %TC and %TN (Section 4.2.2.1, Implication 2; Section 4.3.1.1, Implication 3; Section 4.4.3, Implication 3; Section 4.4.4.1, Implication 3). The seasonal effect on the organic matter contents is only very slight compared to the significant differences in all the parameters along transects of the lochs.

- There was also slight increase in the oxygen uptake rate during the summer from July-September 2002 (Section 3.3.1, Table 3.7), with slight elevation in the $\delta^{13}C$ values from April to June 2002 (Section 3.5.2, Table 3.14), indicating the increase due to contribution of phytoplankton materials.

- For the sediment trap samples, in the summer, there were increases in these parameters: $\delta^{13}C$ values, percentage organic matter due to loss on ignition, %TC and %TN, with slight decrease in the Rp values and C/N ratios, indicating the input of the fresher marine organic matter.

- The %TC at LC0 and LC1 seems to have decreased from the beginning towards the end of the year, while at LC2 and LC5 the %TC increased, most possibly due to transport of materials from the head to the mouth of the loch with time.

4.4.6.2 Transects of the lochs

- The $\delta^{13}C$ values increase from the head to the mouth of both lochs, indicating the presence of more marine organic matter towards the mouths (Figure 3.10).

- Total lignin decreased from the head to the mouth to the outside the lochs. The slight increase of total lignin at LC5 could be due to the accumulation of organic matter here. The slight increase of total lignin at RE6 could be due to the input of terrestrial materials from River Awe.

- The oxygen uptake rates, percentage organic matter due to loss on ignition (LOI), %TC, %TN and %TOC all decreased from the head to the mouth of the lochs (**Appendix 4**). All these indicate the importance of the input of materials from the river sources, and that there is transport of materials along the lochs.

- The oxygen uptake rate was highest at LC1, followed by LC0, LC5, LC3, LC2 and LC6. The inorganic phosphate content also decreased in this order.

- The % labile, % refractory and % total organic matter, as well as the %TC, %TOC and %TN were all highest in locations nearest the river sources, and lowest at LC6 and Camas Nathais in Loch Creran and Loch Etive respectively. This, as well as the significant correlation with total lignin, signifies the importance of the contribution of terrestrial materials from the rivers into the lochs.

- Further down the lochs, the Rp values increase, indicating that the organic matter has become more refractory, due to degradation along the way.

- The Rp values were highest at LC6 and Camas Nathais.

- The C/N ratios were highest at locations nearest the river sources, and lowest at LC6 and Camas Nathais.

- In order to further confirm the importance of the rivers in contributing terrestrial materials into the lochs, the carbon isotope compositions are correlated with distance of each location from the main river source. The distances of the sampling locations from the River Creran and River Etive were measured on an Ordnance Survey map (Table

4.21). All parameters show significant correlation with the distance from the river source. The distribution maps in **Appendix 4** show the mean values of all the parameters along both lochs.

4.4.6.3 The proxies

- Which is the best proxy to determine the biodegradability of sediment organic matter? The best proxy should be sensitive to change, and preferably should be able to be used to identify the biodegradability of the sediment organic matter by itself, and most preferably have significant correlation with other proxies.

- Lignin serves as the biomarker for terrestrial organic matter. There were significant lignin correlations with the percentage organic matter due to LOI, %TC, %TN and $\delta^{13}C$ values, indicating the contribution of terrestrial materials to the organic matter and carbon in the lochs. All these parameters also decreased from the head to the mouth of the lochs, while the $\delta^{13}C$ values increased. Hence terrestrial debris contributes to the organic matter and carbon in the lochs. All these parameters have no significant (regression analysis: $p>0.05$) correlation with the oxygen uptake rates, but the oxygen uptake also decreased from the head to the mouth of Loch Creran (this is explained in Section 4.4.2.3). Hence the biodegradability of the sediment organic matter is generated by the terrestrial organic matter.

- The oxygen uptake rates only correlates significantly (regression analysis: $p<0.05$) with the inorganic phosphate contents, the reason given in Section 4.4.5.

- The $\delta^{13}C$ values correlate significantly with the percentage organic matter due to LOI, %TC, %TN and Rp value. All these indicate the importance of the contribution of terrestrial organic matter from the head to the mouth of the lochs as the marine and terrestrial-derived non-living organic matter can be distinguished on the basis of stable

carbon isotopes compositions due to the large difference (8%) in ^{13}C:^{12}C ratios of atmospheric CO_2 and marine dissolved inorganic carbon (Alperin *et al.*, 1995).

- The best proxy should be able to serve these two purposes mentioned in "Point 1". The proxies which can identify the biodegradability of the sediment organic matter are: oxygen uptake rate, Rp index and C/N ratio.

  The proxies which have significant correlation (simple regression analysis: $p<0.05$) with total lignin and among themselves are: % labile and % total organic matter due to LOI, %TC, %TN, %IP, %OP and Rp index. The most probable reason is because the lignin, %TC, %TN, %IP, and %OP all constitute the % total organic matter. All these also indicate the importance of the contribution of terrestrial materials into the lochs. Among these, only the Rp index is the direct measure of the biodegradability/lability of the sediment organic matter.

- The C/N ratio together with the Rp index, provides a powerful tool to characterize the bulk composition of various biogenic materials at different stages of decomposition (Kristensen, 1990). Kristensen (1990) found that Rp increased from 0.17 to 0.37, and C/N decreased from 87 to 16 during biological decomposition of barley straw, and in marine sediment the Rp increased from 0.43 to 0.47 while C/N decreased from 8.4 to 7.7. In Loch Creran, the Rp increased from 0.43 (at LC0) to 0.56 (at LC5) and 0.64 (at LC6) while C/N decreased from 9.31 (at LC0) to 4.32 (at LC6). In Loch Etive, the Rp increased from 0.40 (at RE2) to 0.75 (at Camas Nathais) and the C/N decreased from 13.60 (at RE2) to 7.57 (at Camas Nathais).

- If the C/N ratio is the direct measurement of the biodegradability of the sediment organic matter, why is the C/N ratio not correlated with other parameters?

  The reason is that the increase or decrease of the C/N ratio could indicate the initial or later degradation stage (see Section 4.4.4.3 for explanations).

- Hence the best proxy to determine the biodegradability of the sediment organic matter is the Rp index. Besides serving as a direct indicator for sediment biodegradability, and has good sensitivity in having significant correlations with many other parameters, the LOI experiment is also a time-saving, economic and easy analytical method.

  The second best proxy is the oxygen uptake rate and the C/N ratio, the latter preferably used together with the Rp index.

  In order to determine that the biodegradability of the sediment organic matter is caused by terrestrial organic matter, these were then correlated with the lignin and $\delta^{13}C$ values. Good correlation indicates that the terrestrial materials play an important role in fuelling the biogeochemical cycling of organic matter in the lochs.

4.4.7 Strengths and weaknesses of past and present studies

- There were previous studies using lignin as the biomarker to determine the distribution of terrestrial organic matter (Section 1.5.1.2), and studies using stable carbon isotopes ratios to trace the relative abundance of terrestrial versus marine organic matter (Section 1.5.2). These studies had concentrated solely on the distribution of lignin or terrestrial and marine organic matter in the coastal and marine environments. The effect of these terrestrial materials on the sediment organic matter was not investigated.

- This is a pioneer study using proxies to determine the biodegradability of sediment organic matter and to determine the effect of terrestrial organic matter on the biodegradability of the sediment organic matter. Lignin as the biomarker for terrestrial organic matter, and the proxies for sediment biodegradability, has successfully carried out these purpose.

4.5 CONTRIBUTION OF TERRESTRIAL ORGANIC MATTER TO TOTAL ORGANIC MATTER

4.5.1 Lignin and Λ versus $\delta^{13}C$ plot

Firstly a total lignin versus $\delta^{13}C$ plot is presented for sediments from Loch Creran so that lignin content and carbon isotope abundances can be compared as tracers of terrestrial-derived organic materials. Such a Λ versus $\delta^{13}C$ plot had been used by Hedges and Mann (1979a and b), and Prahl *et al.* (1994) had used the total lignin (mg/g) versus $\delta^{13}C$ plot. Here the total lignin (mg/g) versus $\delta^{13}C$ plot is used. Only one straight line is plotted so that the $\delta^{13}C$ value of purely marine organic matter, and the total lignin (mg/g) from purely terrestrial materials can be estimated.

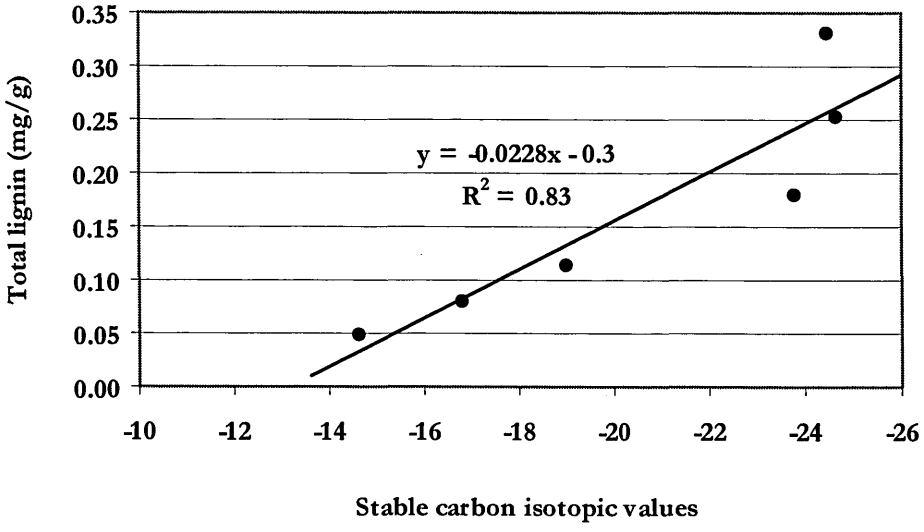
The correlation between lignin and carbon isotope composition in Loch Creran (Figure 4.12a) suggests that the remains of terrestrial materials are thoroughly homogenized during transport so that similar mixtures of land-derived organic materials are deposited over the loch. This close correspondence could not exist if the total lignin and $\delta^{13}C$ values of the land-derived organic material within a depositional zone fluctuated over a wide range (Hedges and Parker, 1976). Extrapolation of the correlation line to total lignin=0 (or substituting total lignin=0 into the equation "y= -0.0228x – 0.3") gives an estimate of the $\delta^{13}C$ value of marine organic matter of –13.16‰. In order to estimate the total lignin of terrestrial-derived organic matter in the sediments firstly a $\delta^{13}C$ value is assigned to this end member (Hedges and Parker, 1976). The mean $\delta^{13}C$ value for all the plant samples analysed is –28.04‰ (from Table 3.14a), and this value is used as the $\delta^{13}C$ value for the terrestrial end-member. When the correlation line is extrapolated to this value (or substituting $\delta^{13}C$= –28.04‰ into the same equation) the average lignin content of 0.35mg/g in the terrestrial organic material is obtained. Hence according to this correlation plot, the $\delta^{13}C$ value for the marine sediment is –13.16‰ and the total lignin in terrestrial organic

matter is 0.35 mg/g. Both –13.16‰ and 0.35mg/g lignin are very close to the mean $\delta^{13}C$ value for LC6 of –14.78‰ and the total lignin of 0.33 mg/g at LC0.

From the correlation line of y= -0.0291x – 0.2558 for Loch Etive (Figure 4.12b), substituting y=0 gives the $\delta^{13}C$ value for the marine sediment of –8.97‰. The very high $\delta^{13}C$ value obtained is due to the high $\delta^{13}C$ value of –11.25‰ at Camas Nathais used to construct this equation. And substituting x= -28.04‰ gives total lignin of 0.56mg/g. These values show that Loch Etive has more terrestrial organic matter compared to Loch Creran. And outside the lochs, Camas Nathais in Loch Etive has higher $\delta^{13}C$ value than LC6 outside Loch Creran. The larger range of values in Loch Etive could be because this loch is the longer loch (Loch Etive is 29.5km and Loch Creran 12.8km in length; Edwards and Sharples, 1986).

From the distribution of the data of both lochs, it seems that these data could also be correlated using an exponential curve, requiring a decision to which of these graphs best suit the purpose.

(a) Loch Creran.



(b) Loch Etive.

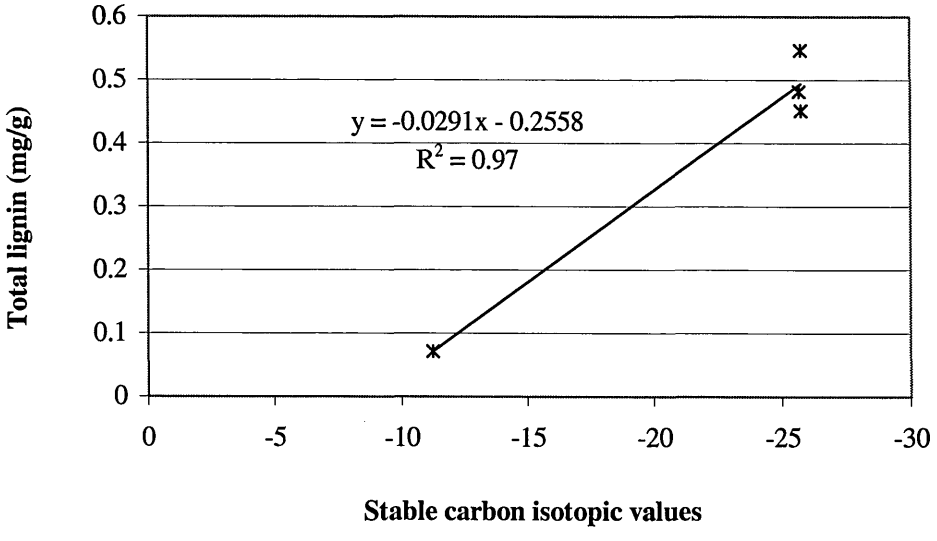
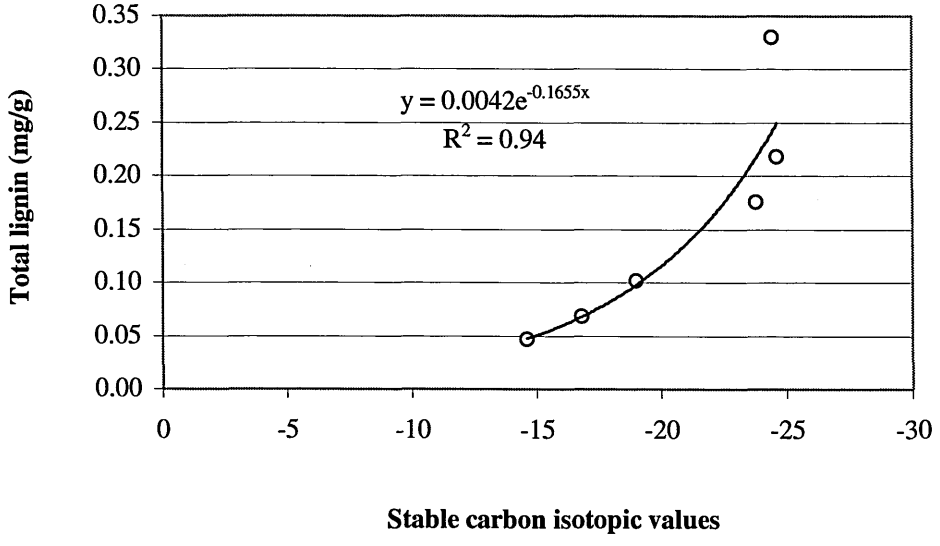


Figure 4.12 Stable carbon isotopic values versus total lignin (linear plots).

(a) Loch Creran.



(b) Loch Etive.

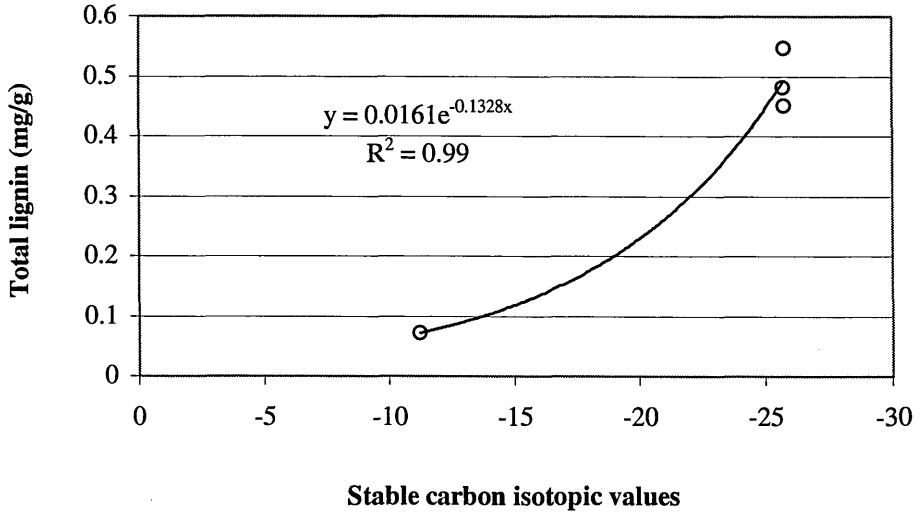
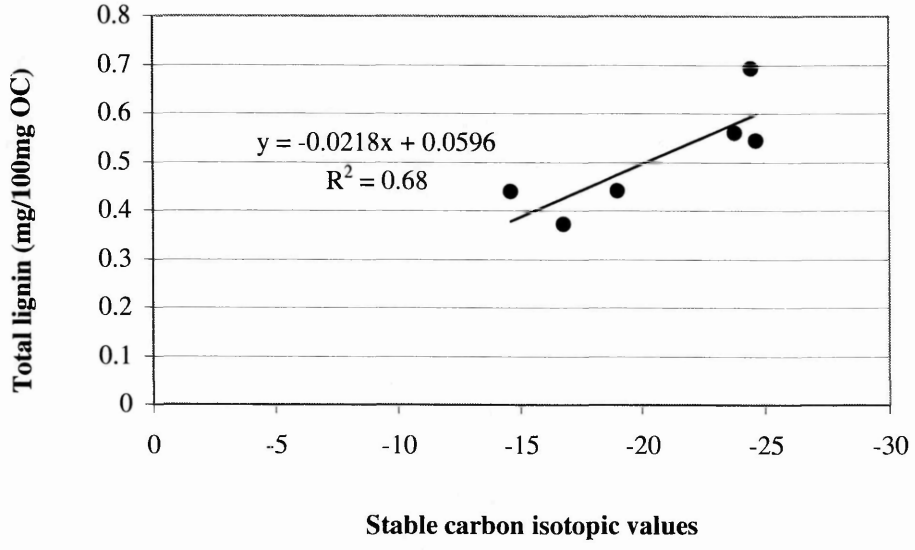


Figure 4.13 Stable carbon isotopic values versus total lignin (exponential plots).

(a) Loch Creran.



(b) Loch Etive.

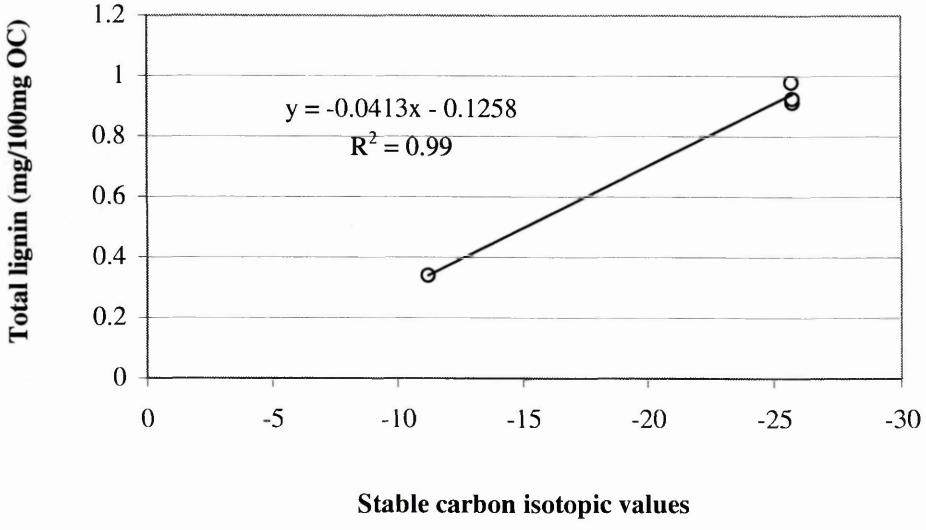
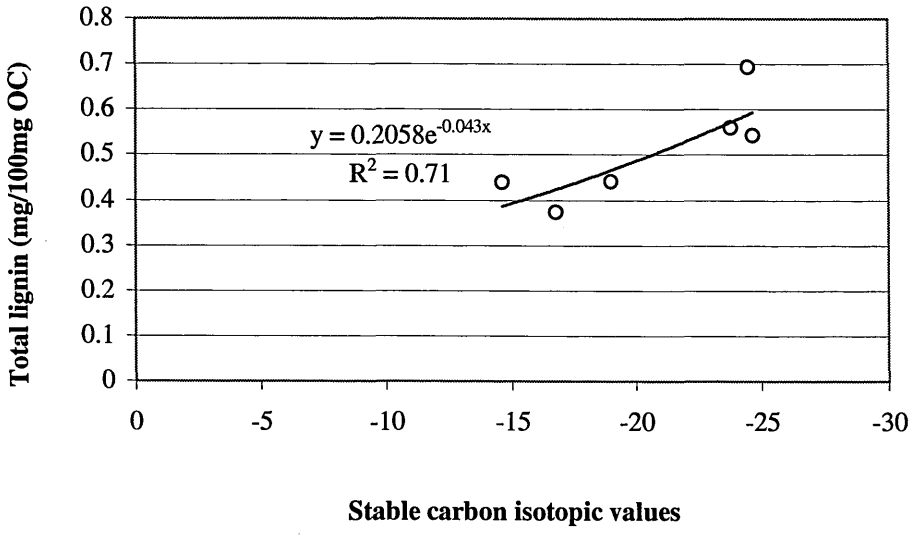


Figure 4.14 Stable carbon isotopic values versus Λ (linear plots).

(a) Loch Creran.



(b) Loch Etive.

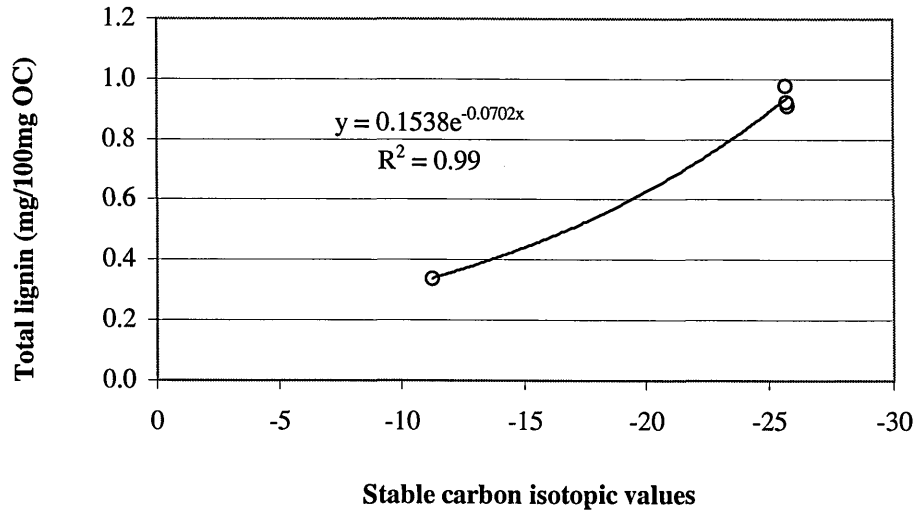


Figure 4.15 Stable carbon isotopic values versus Λ (exponential plots).

Table 4.16 shows the calculation of the $\delta^{13}C$ end-member for marine organic matter and total lignin and Λ end-member for terrestrial organic matter for different graphs. It seems that the total lignin (mg/g) versus $\delta^{13}C$ linear plot is the most suitable plot to determine the $\delta^{13}C$ and total lignin end-members. Firstly, the $\delta^{13}C$ end-member obtained for Loch Creran is –13.16‰, and for Loch Etive –8.79‰. This $\delta^{13}C$ value for Loch Etive is not to be accepted in future calculation as this is out of the range of the $\delta^{13}C$ values for marine organic matter which ranged from –12‰ to -23‰ (see Figure 4.9). Hence in future the $\delta^{13}C$ of –13.16‰ is used as the end-member for marine organic matter for both lochs Creran and Etive. The total lignin end member for both lochs Creran and Etive calculated is 0.34mg/g and 0.56mg/g, respectively. Both values are extremely reasonable, as the mean highest total lignin found at LC0 was 0.33mg/g (refer Table 3.5), and at RE2 was 0.55mg/g (refer Table 3.6).

The total lignin (mg/g) versus $\delta^{13}C$ exponential plot is unable to be used to calculate the $\delta^{13}C$ end-member as "ln 0" is invalid (Table 4.16). Besides, the total lignin end-member calculated for both lochs are inaccurate compared to the actual values. The equations obtained from the Λ versus $\delta^{13}C$ linear plots for both lochs give unacceptable $\delta^{13}C$ end-member values. The Λ values obtained for Loch Creran is 0.67 and for Loch Etive 1.03. In actual results the highest Λ was obtained for LC0 was 0.69, and for RE6 was 0.98. Also there are effectively only two points for Figure 4.15b, and it is not feasible to fit an exponential curve to only two points. Besides, it is assumed that the distribution of marine and terrestrial organic matter is homogenized, hence the linear plot.

Table 4.16 Calculation of the $\delta^{13}C$ and total lignin (mg/g) and Λ end-members from different graphs.

Loch Creran	Loch Etive
Total lignin (mg/g) vs. $\delta^{13}C$ (linear):	Total lignin (mg/g) vs. $\delta^{13}C$ (linear):
Equation: y = -0.0228x – 0.3 (from Figure 4.13a)	Equation: y = -0.0291x – 0.2558 (from Figure 4.13b)
When total lignin = 0, -0.0228x = 0.3 x = -13.16‰	When total lignin = 0, -0.0291x = 0.2558 x = -8.97‰
When $\delta^{13}C$ = -28.04‰, y = -0.0228 (-28.04) – 0.3 = 0.64 – 0.3 = 0.34mg/g	When $\delta^{13}C$ = -28.04‰, y = 0.0291 (-28.04) – 0.2558 = 0.8160 – 0.2558 = 0.56mg/g
Total lignin (mg/g) vs. $\delta^{13}C$ (exponential):	Total lignin (mg/g) vs. $\delta^{13}C$ (exponential):
Equation: $y = 0.0042\ e^{-0.1655x}$ (from Figure 4.14a)	Equation: $y = 0.0161\ e^{-0.1328x}$ (from Figure 4.14b)
When total lignin = 0, Unable to calculate due to : ln 0	
When $\delta^{13}C$ = -28.04‰,	When $\delta^{13}C$ = -28.04‰,
$y = 0.0042\ e^{-0.1655\ (-28.04)}$ = 0.44mg/g	$y = 0.0161\ e^{-0.1328\ (-28.04)}$ = 0.67 (mg/g)
Λ versus $\delta^{13}C$ (linear):	Λ versus $\delta^{13}C$ (linear):
Equation: y= -0.0218x + 0.0596 (from Figure 4.15a)	Equation: y = -0.0413x – 0.1258 (from Figure 4.15b)
When total lignin = 0, -0.0218x = -0.0596 x = 2.73‰ (value not acceptable)	When total lignin = 0, -0.0413x = 0.1258 x = -3.05‰ (not acceptable)
When $\delta^{13}C$ = -28.04‰, y = -0.0218 (-28.04) + 0.0596 = 0.67 (Λ)	When $\delta^{13}C$ = -28.04‰, y = -0.0413 (-28.04) – 0.1258 = 1.03 (Λ)

4.5.2 Terrestrial versus marine fraction

The terrestrial and marine fractions of the sedimentary organic matter can also be calculated using the methods of Mitchell *et al.* (1997) and Tan and Strain (1979). Using the method from Mitchell *et al.* (1997) the stable carbon composition can be used to calculate the composition of organic material at the LC1 and LC6 sediments:

Let, a + b = 1, hence b= 1-a ..... (I),

where a=proportion of phytoplankton detritus, and b=proportion of terrestrial detritus.

Assuming marine organic matter signature = -13.16‰ (this value is obtained from the above calculation in Section 4.5.1).

And terrestrial organic matter signature = -28.04‰ (this value is the mean of the $\delta^{13}C$ values for all the plant samples; Table 3.14a).

At LC0, the mean $\delta^{13}C$= -24.83‰, hence -13.16a + (-28.04b) = -24.83‰ ..... (II)

At LC6, the mean $\delta^{13}C$= -14.78‰), hence -13.16a + (-28.04) = -14.78‰ ..... (III)

Substituting equation (I) to (II),

13.16a + 28.04(1-a) = 24.83

14.88a = 3.21

a=0.22 and b=0.78

Substituting (I) to (III),

14.16a + 28.04(1-a) = 14.78

14.88a = 13.26

a=0.89 and b=0.11

Hence LC0 sediments composed of approximately 22% marine and 78% terrestrial organic matters, and LC6 sediments composed of 89% marine and 11% terrestrial organic matters.

Using the method of Tan and Strain (1979), the fraction of contribution of terrestrial organic carbon (f_t) may be calculated from the following material balance equation:

$$f_t = \frac{\delta i - \delta m}{\delta t - \delta m}$$

Where $\delta i = \delta^{13}C$ of organic carbon in sample

$\delta m = \delta^{13}C$ of marine organic carbon = -13.16‰

$\delta t = \delta^{13}C$ of terrestrial organic carbon = -28.04‰

Hence for the LC0 sediment where the $\delta^{13}C$ = -24.83‰,

$$f_t = \frac{-24.83 - (-13.16)}{-28.04 - (-13.16)} = 0.78$$

Hence the two methods give the same results for the fractions of organic matter. The calculated percentages of terrestrial and marine organic matter for each location are given in Table 4.17. The most important implication here, however, is that the location nearest the river input was composed of mostly terrestrial organic matter, and further down the loch, the marine organic matter predominates.

Table 4.17 Percentages of terrestrial and marine organic matter. The percentage fractions of terrestrial and marine organic matter are calculated using end-member for Loch Creran as –28.04‰ and –13.16‰, and for Loch Etive –28.04‰ and –13.16‰. The % terrestrial organic matter for Camas Nathais is calculated by subtracting –13.16‰ by –11.25‰.

Location	% terrestrial organic matter	% marine organic matter
LC0	78	22
LC1	69	31
LC2	73	27
LC3	23	77
LC5	52	48
LC6	11	89
RE2	84	16
RE5	85	15
RE6	85	15
Camas Nathais	13	87

All these indicate that terrestrial materials predominate in locations in the upper lochs, whilst further down the loch, marine organic matter predominates.

4.5.3 % lignin to TOC and TOM

4.5.3.1 % lignin to TC and TOC

Bader (1956) calculated the % lignin to total carbon (TC) as "lignin carbon/TC x 100%". An example of the calculation is shown here.

At LC0, mean total lignin = 0.33mg/g and mean %TC = 5.10%

In 1g of sediment, there is 5.10/100 x 1g = 0.051g TC

Hence % total lignin to TC = 0.33 x 10^{-3}g/0.051g x 100% = 0.65%.

Similar calculation was carried out with %TOC, and the results are given in Table 4.18.

%TOC = 4.77%

In 1g sediment, there is 0.0477g TOC.

% lignin to TOC = 0.33 x 10^{-3}g/0.0477g x 100% = 0.69%

The % lignin to TOC is equal to Λ (mg/100mg OC).

Table 4.18 Percentage lignin to TC and TOC.

Location	Total lignin (mg/g)	Λ (mg/100 mgOC)	%TC	%TOC	% lignin to TC	% lignin to TOC
LC0	0.33	0.69	5.10	4.77	0.65	0.69
LC1	0.22	0.54	4.57	4.01	0.48	0.55
LC2	0.18	0.56	3.58	3.14	0.50	0.57
LC3	0.07	0.37	2.67	1.83	0.26	0.38
LC5	0.10	0.44	3.27	2.30	0.31	0.43
LC6	0.05	0.44	1.92	1.07	0.26	0.47
RE2	0.55	0.91	6.26	6.02	0.88	0.91
RE5	0.45	0.92	5.56	4.88	0.81	0.92
RE6	0.48	0.98	5.77	4.93	0.83	0.97
Camas Nathais	0.07	0.34	3.49	2.12	0.20	0.33

Overall the contribution of lignin to TC and TOC do not exceed 1%. The contribution of lignin to TC and TOC is highest in locations near the river sources and decreases further down the loch. At LC0, lignin contributed 0.65% to TC, and 0.69% to TOC. At LC5, the contribution of lignin to TC and TOC decreased to 0.31% and 0.43%, respectively. At LC6, these decreased to 0.26% TC and 0.47%, respectively. In Loch Etive, the % contribution of lignin to TC and TOC are 1 to 3 times higher than in Loch Creran. The highest contribution of lignin to TC and TOC occur in locations within the loch. The percentage contribution of lignin to TC ranged from 0.81 to 0.88%, and percentage contribution of lignin to TOC from 0.91 to 0.97%. At Camas Nathais, percentage contribution of lignin to both TC and TOC decreased significantly to 0.20% and

0.33%, respectively. All these indicate the importance of the contribution of river input of lignin material to the lochs, and the transport of this material along the loch transect towards the sea.

4.5.3.2 % lignin and terrestrial organic matter to total organic matter

Here the contribution of lignin and terrestrial organic matter to the TOM is investigated. By using results from LC0, examples of calculations are shown here.

At LC0, the mean %TOM = 17.11%

In 1g of sediment there is 17.11/100 x 1g =0.17g TOM

The mean % labile OM = 9.83% and % refractory OM = 7.28%

Hence in 1g of sediment, there is 9.83/100 x 1g = 0.10g labile OM

In 1g of sediment, there is 7.28/100 x 1g = 0.07g refractory OM

The terrestrial OM fraction at LC0 is 78%.

Hence in 0.17g TOM, there is 78/100 x 0.17g = 0.13g terrestrial OM

0.04/0.17 x 100% = 23% marine OM

% lignin to terrestrial OM = 0.33 x 10^{-3}g/0.13g x 100% = 0.25%

Table 4.19 Percentages lignin and terrestrial organic matter to total organic matter.

Location	%TOM	% labile OM	% refrac OM	% terres OM	% marine OM	TOM (g)	Labile OM (g)	Refrac OM (g)	Terres OM (g)	% terres to TOM (%)	% lignin to terrestrial OM
LC0	17.11	9.83	7.28	78	22	0.17	0.10 (58.82%)	0.07 (41.18%)	0.13	76.49	0.25
LC1	16.19	9.34	6.88	69	31	0.16	0.09 (56.26%)	0.07 (43.75%)	0.11	68.75	0.20
LC2	12.76	6.61	6.15	73	27	0.13	0.07 (53.85%)	0.06 (46.15%)	0.10	76.92	0.18
LC3	8.38	3.47	4.91	23	77	0.08	0.03 (37.50%)	0.05 (62.50%)	0.02	25	0.35
LC5	6.53	3.43	3.09	52	48	0.06	0.03 (50%)	0.03 (50%)	0.03	50	0.33
LC6	9.63	3.60	6.03	11	89	0.10	0.04 (40%)	0.06 (60%)	0.01	10	0.50
RE2	17.69	10.56	7.13	88	12	0.18	0.11 (61.11%)	0.07 (38.89%)	0.16	88.89	0.34
RE5	20.28	10.88	9.40	88	12	0.20	0.11 (55%)	0.09 (45%)	0.18	90	0.25
RE6	22.80	11.68	11.12	88	12	0.23	0.12 (52.17%)	0.11 (47.83%)	0.20	86.96	0.24
CN	15.11	3.04	12.08	12	88	0.15	0.03 (20%)	0.12 (80%)	0.02	13.33	0.35

Note: terres=terrestrial; TOM=total organic matter. By giving TOM as 100% composition, the percentage of labile and refractory organic matter are calculated and given in parentheses. The % terrestrial OM and % marine OM are calculated in Section 4.6.2. See text for the calculation of TOM (g), labile OM (g) and refractory OM (g).

From Table 4.19, by giving 100% composition for the total organic matter, the percentages of labile and refractory organic matter composition of the total organic matter are calculated. The proportion of labile organic matter decreased from the head to the mouth and outside the lochs, while the refractory organic matter increased.

The contribution of terrestrial organic matter to total organic matter also decreased from the head to further down the lochs.

That the percentage contribution of lignin to the total terrestrial organic matter increased further down the lochs shows that this lignin material is the fraction remains while the other more labile fraction of the terrestrial organic matter was being degraded along the lochs. Hence further down the loch, the ratio of the lignin to the total terrestrial material increased, as young carbon is degraded over residence times of river and coastal waters, leaving an older and more refractory component for oceanic export (Raymond and Bauer, 2001).

4.5.4 Summary

1. In Loch Creran at LC0, the % total lignin contribution to TC and TOC are 0.65% and 0.69%. This decreased to LC6 where lignin contributes 0.26% TC and 0.47% TOC.
2. In Loch Etive at RE2, lignin contributes 0.88% of TC and 0.91% of TOC. This decreased to Camas Nathais where lignin contributes 0.20% of TC and 0.33% of TOC.
3. The contribution of terrestrial to total organic matter also decreased further down the loch.
4. The lignin material being the more refractory fraction, the percentage of lignin to total terrestrial organic matter increased further down the lochs.
5. All these indicate the importance of the rivers in contributing terrestrial organic matter into the lochs.

4.5.5 Strengths and weaknesses of past and present studies

In this work the reason behind the usage of the total lignin (mg/g) versus $\delta^{13}C$ values plot is given. Also the percentages of lignin to total organic matter, total carbon and terrestrial organic matter were determined.

4.6 WHAT HAPPENS TO TERRESTRIAL ORGANIC MATTER IN SEA LOCHS?

4.6.1 Loch Creran

Using Loch Creran as the model, the transport of terrestrial materials into and out of the loch, and the transport within the loch will be considered. The input of freshwater at the head of Loch Creran transports terrestrial debris from River Creran catchment area into the loch. A higher rate of decomposition observed at the head of the loch compared to further down is more likely due to higher degradability of the newly settled organic matter, and this organic matter consists of materials of terrestrial origin (Section 4.4.2.2: Implications 1 to 5).

Once in the loch, terrestrial debris is transported via several mechanisms. Some materials are transported out of the loch via the less dense out-flowing freshwater, above the denser incoming seawater. These materials consist mostly of the non-woody tissues closely associated with the finer particles. The denser particles sink to the bottom water and onto the surface sediments. The shear forces between the incoming denser saline seawater and the out flowing less dense freshwater cause material resuspension in the water column. The incoming denser saline water can also cause material resuspension from the sediment surface.

4.6.1.1 Hydrodynamic sorting

The increase of C/V ratios from the head to the mouth of Loch Creran indicates the increase in the non-woody tissues further down the loch. Non-woody tissues being the more fragile fraction, forms small particles when broken down mechanically and remain in suspension to be transported offshore and accumulate in shelf sediments. The coarser and denser organic matter containing water-logged C3 vascular plant detritus with higher woody angiosperm and gymnosperm tissues, is retained in bays, estuaries and the inner shelf along with the coarser silt and sand-sized mineral particles (Prahl, 1985; Bird and Pousai, 1997; Goni *et al.*, 1997 and 1998; Keil *et al.*, 2002; Miltner and Emeis, 2000; Bianchi *et al.*, 2002). Also this hydrodynamic

processes act to trap the coarse, lignin-rich woody plant debris in the shelf environments and allow other finer, lignin-poor riverine particles to advect further offshore (Prahl *et al.*, 1994).

4.6.1.2 Particles in the water column

The higher % labile, % refractory and % total organic matter, as well as %TC and %TN, and total lignin in the sediment trap samples compared to the LC1 surface sediments all indicate the greater abundance of organic matter and carbon in sedimenting particles in the water column than in surface sediments (see Table 4.22). The decrease of organic matter when reaching the surface sediments indicates that some materials have been remineralised during sedimentation.

The Rp value and C/N ratio in the LC1 sediments (Rp=0.42, C/N=9.30) are higher compared to the sediment trap samples (Rp=0.38, C/N=8.41). The higher C/N ratio indicates that surface sediments have been more metabolised due to preferential uptake of nitrogen by bacteria (Bianchi and Argyrou, 1997) during organic matter degradation. The higher Rp value at LC1 surface sediment shows that there was a greater fraction of refractory organic matter present at LC1, as some of the labile fraction of the organic matter has undergone degradation in the water column.

Both the total lignin (mg/g) and (Ad/Al)v values were found to be higher in the sediment trap compared to LC1 surface sediment, most probably due to association of the lignin material with the buoyant suspended sediment (Reeves and Preston, 1991).

4.6.1.3 Surface sediments

Surface sediments at individual locations showed extremely constant organic matter contents. All the experimental parameters: lignin, oxygen uptake rates, carbon isotope composition, percentage organic matter due to loss on ignition (LOI), and the %TC and %TN and C/N ratio at individual locations exhibited no consistent change with time. The LOI is the experiment carried

out for the longest period of time and during this period there were no significant trends in the changes in the LOI results (Section 3.4.3, Table 3.11). Hence much of the organic matter decomposition takes place near the sediment-water interface (Ingall and Van Capellen, 1990; Henrichs, 1992) and the organic matter preserved in sediments is totally refractory when deposited and survives diagenesis with little change (Henrichs, 1992).

In Loch Creran, total lignin and (Ad/Al)v were sometimes higher at the 0-1cm than the 9-10cm sediment layer (Table 4.20). Another interesting finding is that in Loch Etive, the Rp values are always higher in the 9-10cm layers than the 0-1cm layers (see also Table 3.11h). In Loch Creran however, the Rp values were sometimes higher in the 0-1cm sediment layers. The explanation is also given in Section 4.2.2.1 (Implication 4).

The fresher materials trapped in the 9-10cm layer could be due to rapid sedimentation rate in Loch Creran (Rowe and Gardner, 1979; Requejo *et al.*, 1986). With time the sediment in the 9-10cm layer will have been subject to degradation. For example, the (Ad/Al)v for the sediment from LC1 collected on June 2002 was higher in the 0-1cm than 9-10cm sediment layer. With time, the lignin material will also have undergone degradation, as indicated by the increase of the (Ad/Al)v ratio in the 9-10cm compared to the 0-1cm sediment layer in the next sampling month in September 2002.

The more degraded materials, as seen from the higher Rp values in the surface sediments, could be due to deposition of the highly degraded suspended particles.

The reason this trapping of fresher materials deeper in the sediments did not occur in Loch Etive, is due to the difference in the hydrodynamic regime between the two lochs.

Table 4.20 Experimental parameters for sediment depth profiles. The ">" and "<" symbols here do not indicate significantly higher or lower than, but just to indicate more or less than between the 0-1cm and 9-10cm layers.

Lignin parameter	Locations and sediment depth.																	
	Creran head		LC1 (4.6.02)		LC1 (2.9.02)		LC1 (12.12.02)		LC0 (14.11.02)		LC2 (2.9.02)		LC3 (30.9.02)		LC5 (12.12.02)		LC6 (30.9.02)	
	0-1cm	9-10cm	0-1cm	9-10cm	0-1cm	9-10cm	0-1cm	9-10cm	0-1cm	9-10cm	0-1cm	9-10cm	0-1cm	9-10cm	0-1cm	9-10cm	0-1cm	9-10cm
Lignin	0.0684 >	0.0437	0.2721 >	0.2147	0.2704 >	0.1952	0.2719 <	0.3163	0.3195<	0.3263	0.1204 <	0.1687	0.0799 >	0.0766	0.1072>	0.0536	0.0691>	0.0294
(Ad/Al)v	0.5918 >	0.4894	1.1571 >	0.9500	0.7195 <	0.8889	0.8185 <	0.8603	0.8651<	1.3551	0.7083 <	0.8780	1.5758 >	1.4324	0.5611>	0.5077	0.8235<	0.9487
S/V	0.3197 >	0.2595	0.6673 >	0.6547	0.5645 <	0.6135	0.6257 >	0.5967	0.5886>	0.5832	0.4382 <	0.5802	0.5906 >	0.5195	0.4948>	0.3868	0.2755<	0.4129
C/V	1.2230 >	1.1027	0.8131 <	1.0025	0.7909 <	0.9617	0.6307 <	0.6610	0.6503>	0.5621	0.6890 <	0.8790	0.5066 >	1.4727	0.7339<	1.1415	1.3321>	0.4839
$\delta^{13}C$	-25.04>	-23.86	-23.13		-23.20<	-24.33	-23.98<	-24.57	-24.45<	-24.28			-17.07<	-18.59			-14.38>	-14.30
LOI:																		
%labile					12.70>	12.45	10.41>	9.18	10.25>	8.78			4.77>	3.77	1.58<	1.65		
%refrac					7.84>	6.57	8.56>	7.40	6.15>	4.83			6.02>	4.64	2.57>	1.72		
%TOM					20.54>	19.02	18.97>	16.58	16.40>	13.61			10.79>	8.41	4.16>	3.37		
Rp					0.38>	0.35	0.45	0.45	0.38>	0.36			0.62>	0.58	0.62>	0.51		
%TC									4.37>	4.05	2.93<	3.32	1.60<	1.90				
%TN									0.45>	0.38	0.32<	0.37	0.24<	0.27				
C/N									9.71<	10.66	9.16>	8.97	6.67<	7.04				

4.6.1.4 Effect of bioturbation

The total lignin, (Ad/Al) values, %TC, %TN, C/N, and even $\delta^{13}C$ values were at times higher or lower in the 0-1cm or 9-10cm layers (see Table 4.20). Besides indicating rapid sedimentation rate, this also indicates the effect of bioturbation in the sediments from Loch Creran.

The effect of oxygen in degrading the sediment organic matter is also important (Section 1.2.3.2). Even brief, periodic re-exposure to oxygen results in more complete and sometimes rapid decomposition than is possible under constant conditions and most benthic organic carbon decomposition takes place within the bioturbated zone of sediments underlying oxygenated waters (Aller, 1994). The net anoxic mineralization rates of organic matter also increase with increasing macrofaunal burrow spacing and irrigation intensity (Aller and Aller, 1998). The increase in decay capacity is partly caused by injection of oxygen into the sediment, enhancing the decay of old, oxygen sensitive organic matter several fold (Kristensen, 2000).

4.6.1.5 Effect of fish farms and other farms

The possibility of the effect of fish farms on the quality of the sedimentary organic matter in our sampling locations is also considered (Section 1.1.1). As all the locations, except LC5, are located quite far from any farm, any possible effect of the fish farms on the organic matter quality in our sampling locations is negated. Brown *et al.* (1987), Ye *et al.* (1991), Holmer and Kristensen (1992), Nickell *et al.* (2003), and Pereira *et al.* (2004) all observed the effects of fish farms on the sediment organic matter and benthic fauna were limited to the immediate vicinity (within 30m) of the cages (Table 4.21).

The only station that could be influenced by a shell fish farm is LC5. The nearest shell fish farm was located 350m from LC5. Hence if there was organic enrichment at LC5, this effect was only slight, as there was only slight elevation of the %TC, %TN and only a slight increase in the oxygen uptake rate. The higher lignin content, as well as other organic matter parameters at LC5,

could also be due to the accumulation of the terrestrial debris at LC5, as this is located in a sheltered bend. The seaweed-processing plant that used to be located at Barcaldine (Tett and Wallis, 1978) was too far to contribute much to the organic input to all the stations in Loch Creran. Figure 4.16 shows all the fish farms and shell fish farms along both lochs Creran and Etive around the year 2000 to 2002 [this information was obtained from the Marine Science (SEPA), Dingwall]

Table 4.21 Previous studies on the effect of fish farms.

Year	Authors	Findings
1987	Brown *et al.*	Observed slightly enriched zone at <25m with reduced oxygen level, and low total biomass in sediments from Loch Spelve, but a 'clean' zone at distances >25m.
1987	Gowen and Bradbury	The effect of enrichment from a farm which include organic carbon and organic nitrogen, ammonia, urea, bicarbonate, phosphate, vitamins, therapeutants and pigments in increase in the oxygen consumption by heterotrophic organisms within the sediments
1991	Ye *et al.*	Observed that underneath a farm in Tasmania contained very few species of macrobenthos, but beyond 30m the community appears undisturbed.
1992	Holmer and Kristensen	Found that a control location 30m away from a fish farm in Koding Fjour, Denmark appear undisturbed. Also found sediment metabolism beneath the cages was about 10 times higher during farming period (525 to 619 mole CO_2 m^{-2} d^{-1}) than at an unaffected station (24 to 70 mole CO_2 m^{-2} d^{-1}), hence organic enrichment and increase in the oxygen uptake rates were observed in sediment near cages
2003	Nickell *et al.*	Observed an increased in the oxygen uptake rates (etc.) beneath a fish farm, but further from this location declined.
2004	Pereira *et al.*	Found that the percentages of organic carbon in the sediment (0-3cm) decreased with distance from the fish farm site, being two to three times higher at the edges of the cages than 30m away.

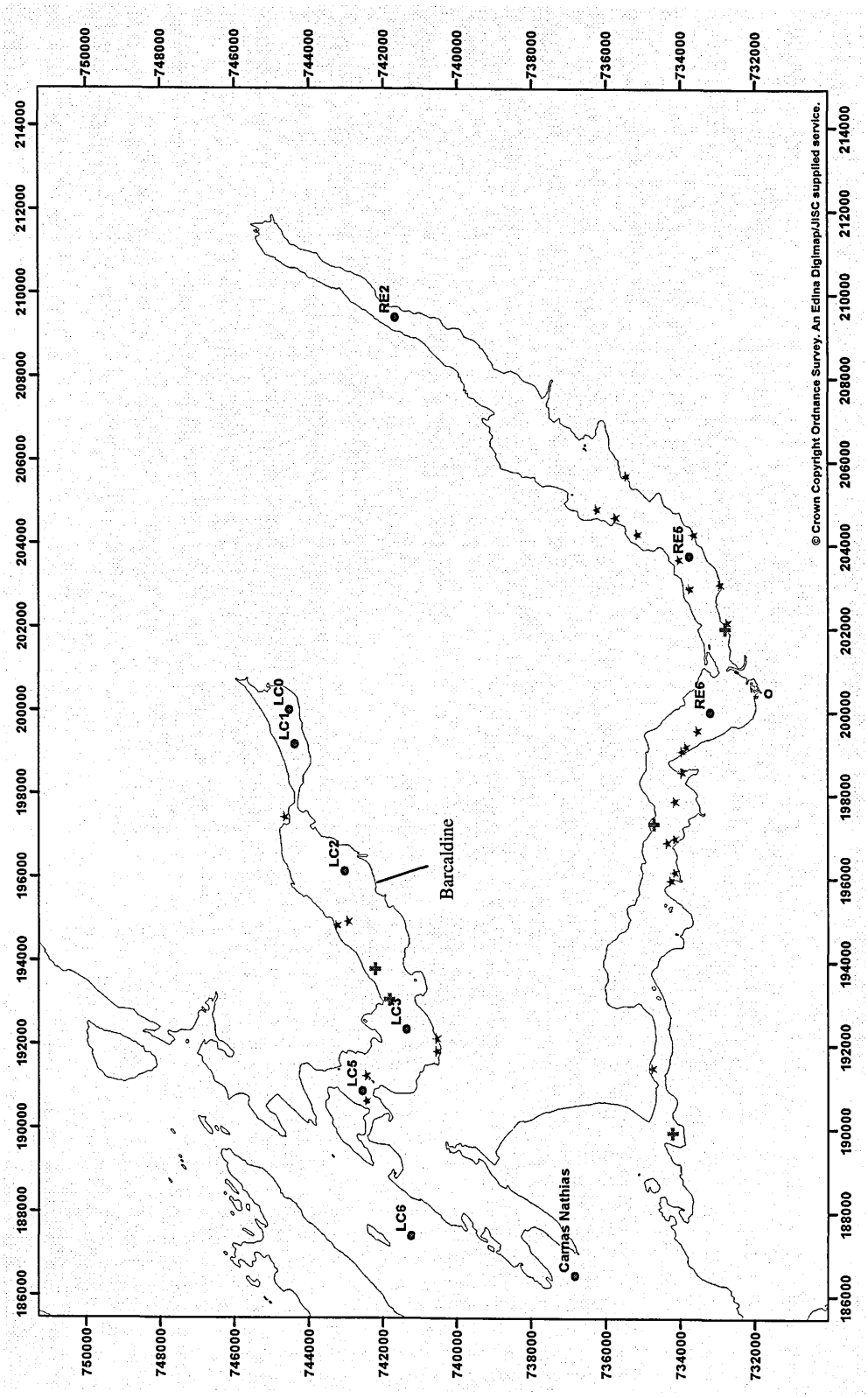


Figure 4.16 Locations of fish farms and shellfish farms in Loch Creran and Loch Etive.

Farms locations and coordinates are obtained from the SEPA, Dingwall. These farms were present in these locations from 2000 to 2002.

Keys: Cross indicates fish farms, and star indicates shell fish farms.

Loch Etive

Marine Fish Farms

Kames Fish Farming – Inverawe: NN 0200 3280
Mainstream Scotland Ltd - Ardchattan Bay: NM 9730 3470
Mainstream Scotland Ltd - Camas Bruaich: NM 8990 3420

Shell Fish Farms

Rubha Mor: NM 921 405
South Shian Bay 2: NM 906 424
c/o Creagan Farm: NM 974 446
South Ardnaclach Farm: NM 948 432
Loch Creran: NM 918 405
Druim na Claoidh: NM 949 429
South Shian Bay 1: NM 912 424

Other discharges

Final Effluent Taynuilt: NN 0047 3163

Loch Creran

Marine Fish Farms

Scottish Seafarms - Creran A:	NM 9307 4182
Scottish Seafarms - Creran B:	NM 9380 4220

Shellfish Farms

Achnacloich:	NM 961 341
Achnacloich - (Abbots):	NM 968 343
Cadderlie Bay:	NN 048 362
Site 2, Loch Etive:	NM 959 342
Craig Point:	NN 036 340
Airds Bay:	NM 995 335
Lorn Fisheries, Inverawe:	NN 021 327
Inverawe:	NN 029 337
Mouth of Loch Etive:	NM 914 347
Glen Noe:	NN 042 336
Craig Bay:	NN 042 351
Port Mor:	NN 046 357
Goosebay:	NM 985 339
Glenoe Site:	NN 056 354
Sgeir nan Mine:	NM 978 341
Port Na Mine:	NN 030 329
Rubha Ban:	NM 969 341
Site 6, Loch Etive:	NM 991 338
Airds Bay:	NM 990 339

Other discharges

Final Effluent: Hydro Seafood GSP Ltd, South Shian	NN 9103 4179

4.6.1.6 Flocculation

During transport, particles in the lochs are subjected to important changes in size as well as composition; the two most important processes are flocculation and biological grain-size changes.

(i) Flocculation:

Most of the coarser material is fine sand, sometimes mixed with shell remains. The finer material is composed of clays, hydroxides and organic matter. Since particles are largely trapped in estuaries their residence time is generally much longer than that of the estuarine water mass, even if they remain in suspension. Particles in suspension are removed with the water as it is exchanged. Clay particles and hydroxides are flocculated by the inorganic salts of seawater, mostly already at very low salinities. In quiet water the flocculates can slowly grow to sizes of several hundred microns. If the water is agitated growth can be faster because of more frequent particle collisions, but at the same time this agitation limits the final size of the flocculates. (Postma, 1980). Flocculation of river colloidal material by seawater electrolytes in the 0-5‰ salinity range is a common process to all estuaries (Billen *et al.*, 1990).

(ii) Changes in grain-size by biological activity can take place in several ways. New particles are added to sediment assemblage by the growth of bacteria and phytoplankton. The macrobenthos in estuaries, specifically suspension and deposit feeders, influence grain-size in two opposite directions. First, small particles are combined to larger ones in faecal pellets. These causes an increase in grain-size comparable with flocculation, but since faecal pellets can be very stable the reverse process proceeds much slower than in the case of chemical flocculation. The ultimate effect of faecal pellet formation is increased stability and cohesion of the estuarine deposits (Postma, 1980).

Deposit feeders rework the deposit, decreasing compaction and increasing water content and giving a rough texture to the sediment-water interface. As a result, bottom water is

easily resuspended even by weak tidal currents. Besides biological activity, new particles can be added to the suspended matter assemblage by flocculation of dissolved matter (Postma, 1980).

In Loch Creran the flocculation could have occurred due to these processes:

1. The mixture of fresh and saline waters is more common, and this induces flocculation.
2. There is entrainment of the less dense out flowing fresh water with the denser incoming saline water, creating a layer of permanently suspended particles. This provides longer residence time for the particles in suspension, enabling the flocculation process to occur.
3. The much agitation of the water will limit the sizes of the floccules.
4. The activities of the macro benthos will create new particles, giving the rough texture of the water-sediment interface. Eventually these particles will be resuspended due to the changing water.
5. Flocculation also occurs during mixing of water due to wind effects.

In Loch Etive, flocculation most probably occurs due to these processes:

1. There is less movement between the fresh and saline water, due to the larger amount of fresh water input. Also there is a longer period of separation between the fresh and saline water. These conditions do not facilitate the flocculation process.
2. The flocculation process most probably is facilitated by the wind mixing effect causing water mixing and material resuspension.
3. Similarly to Loch Creran, the macrobenthos will also play the role in creating new particles due to formation of feacal pellets. These particles most probably are disassemblaged during periods of water renewal.

4.6.2 Loch Creran versus Loch Etive

The differences in hydrological and hydrodynamic regimes between Loch Creran and Loch Etive (Section 1.3.2) result in differences in the quality and quantity of organic matter between these two lochs (Table 4.21).

4.6.2.1 The hydrographic regimes

Loch Creran has a surface area of 13.53km^2 (Gage, 1972), and catchment area of 164km^2 (Edwards and Sharples, 1986; Section 1.3.2.1). Hence the surface to catchment area ratio of Loch Creran is 12.12. Loch Etive has a surface area of 28.29km^2 (Gage, 1972) and catchment area of 1400km^2 (Edwards and Sharples, 1986; Section 1.3.2.2). Hence the surface to catchment area ratio of Loch Etive is 49.49. This means that Loch Etive receives approximately four times greater input of terrestrial material per unit area compared to Loch Creran. As a result, the total lignin content, as well as TC, TOC, and total organic matter, in Loch Etive were higher compared to Loch Creran. The more negative $\delta^{13}C$ values in Loch Etive also indicate more abundance of terrestrial materials (Table 4.22).

4.6.2.2. The hydrodynamic regimes

Loch Creran

As Loch Creran is shallower and smaller (maximum depth 45.5m; 13.53km^2) than Loch Etive (28.29km^2, maximum depth 153m), there is no modification of the tidal cycle in Loch Creran as occurs in Loch Etive. In Loch Creran, runoff effects are slight, but tidal flushing is sufficient to ensure ventilation of the bottom water. When the influence of tidal mixing relative to that of the river flow is very strong, the loch becomes well mixed, with very little variation in salinity or mean current with depth (Bowden, 1980).

Sediment is introduced into the upper loch by the river and vigorous mixing exchanges sediment into the upper layer. Sediment settles into the lower layer in areas of less vigorous mixing and joins sediment entering from the sea on the landward residual flow. It then travels back towards the head of the loch (after Dyer, 1969). This action forms a very effective sorting mechanism (Schubel, 1969), resulting in the hydrodynamic sorting process occurring in this loch.

Loch Etive

Because Loch Etive is the larger and deeper loch, the isolation in the inner basin of the loch by a series of shallow sills, combined with the high input of freshwater, prevents the continuous exchange of marine bottom water and leads to salinity stratification. Between periods of renewal, the bottom waters become depleted in oxygen but they do not become anoxic (Murray *et al.*, 2003). A bottom water renewal is caused by low freshwater runoff. The water above sill level in the deepest basin usually has low salinity because of locally high freshwater runoff. When the runoff lessens, salinity and density rise and the loch bottom water are renewed by the density current inflow. When the runoff rises again, the new water is isolated by its high density and it stagnates for many months (Edwards and Edelsten, 1977). The water above sill level in the deepest basin usually has low salinity because of the locally high freshwater runoff. When the runoff lessens, salinity and density rise and the bottom water of the loch are renewed by the density current inflow. When the runoff rises again, the new water is isolated by its high density and it stagnates for many months. During stagnation, the estuarine develops circulation develops in the upper thirty or so meters with a primary pycnocline* separating the outgoing brackish water from the incoming sea water. The bottom water is separated from the estuarine circulation by a secondary pycnocline which inhibits mixing and turbulent transfer. The temperature and salinity change slowly, and the density falls slowly, priming the system for the next renewal (Edwards and Grantham, 1986).

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* **pycnocline** layer in which the density of the water rapidly increases with depth, because of the presence of a halocline or a thermocline or both (Baretta-Bekker *et al.*, 1998).

Hence in Loch Etive, there is no hydrodynamic sorting process as occurred in Loch Creran. The differences between the two lochs result in differences in the C/V ratios: the C/V ratios along Loch Creran increased but in Loch Etive the C/V decreased further down the loch. In Loch Creran, hydrodynamic sorting occurred whereas in Loch Etive the lignin phenols still undergo diagenesis.

Table 4.22 All the experimental parameters for Loch Creran and Loch Etive.

Location	Depth (m)	Distance from the river mouth (m)	Lignin (mg/g)	Λ (mg/g)	$\delta^{13}C$	oxygen uptake rate	% labile OM	% refrac OM	%TOM	Rp	%TC	%TOC	%TN	OC/N
LC0	15.42	610	0.3305	0.6929	-24.83	17.60	9.83	7.28	17.11	0.43	5.10	4.77	0.52	9.31
Sediment trap	10	720	0.3041		-21.33		14.75	8.69	23.44	0.38	6.38		0.78	8.41
LC1	37	720	0.2180	0.5436	-23.44	20.78	9.34	6.88	16.19	0.42	4.57	4.01	0.47	8.26
LC2	27	4 150	0.1760	0.5605	-23.99	9.40	6.61	6.15	12.76	0.48	3.58	3.14	0.38	8.25
LC3	49	8 150	0.0682	0.3727	-16.59	12.44	3.47	4.91	8.38	0.59	2.67	1.83	0.28	6.60
LC5	27	10 100	0.1016	0.4417	-20.96	15.13	3.43	3.09	6.53	0.56	3.27	2.30	0.34	6.65
LC6	48.94	16 000	0.0470	0.4393	-14.78	9.44	3.60	6.03	9.63	0.64	1.92	1.07	0.24	4.32
RE2	37	4400	0.5477	0.9098	-25.71		10.56	7.13	17.69	0.40	6.26	6.02	0.44	13.60
RE5	123	14150	0.4509	0.9240	-25.75		10.88	9.40	20.28	0.46	5.56	4.88	0.50	9.76
RE6	57	18360	0.4823	0.9783	-25.76		11.68	11.12	22.80	0.39	5.77	4.93	0.54	9.08
Camas Nathais		33400		0.3387	-11.25		3.04	12.08	15.11	0.75	3.49	2.12	0.28	7.57

4.6.3 Summary

1. In Loch Creran, the hydrodynamic sorting process occurring results in non-woody tissue transported further down the loch, hence the increase of C/V ratios further down the loch.

2. The high sedimentation rate results in fresher materials trapped in the 9-10cm sediment layer.

3. Bioturbation enables the degradation of organic matter within the 0 to 10cm sediment layer.

4. The more highly degraded material found at times at the surface 0-1cm compared to the 9-10cm sediment layer could be due to deposition of the more highly degraded suspended materials.

5. The larger catchment to loch area ratio for Loch Etive results in higher lignin, organic matter and total carbon inventories compared to Loch Creran.

6. In Loch Etive, sediments were not subject to hydrodynamic sorting due to water stratification for some period of time.

4.7 CARBON BUDGETS

4.7.1 Organic matter and carbon fluxes in Loch Creran

The total fluxes of organic matter, total carbon and total lignin are calculated based on the average sedimentation rate measured for the sediment trap samples. The mean sedimentation rate for the sediment trap is 11.11g of dry sediment weight/m^2/d (obtained from Section 3.1.2). The mean values for TC, total organic matter due to loss on ignition (LOI), and total lignin for individual locations in Loch Creran are multiplied with the mean sedimentation rate, 11.11 $gm^{-2}d^{-1}$. As a result the fluxes calculation for all locations in Loch Creran are only estimation based on this mean sedimentation rate. Examples of the calculation of fluxes are shown below (also see Rowe and Gardner, 1979; and Chester and Larrance, 1981 for calculation of fluxes). The calculations for all fluxes for individual locations are similar. Results are given in Table 4.23.

The mean total lignin at LC0 is 0.3305mg/g.

Hence the mean lignin flux at LC0 = 0.3305mg/g x 11.11 g/m^2/day = 3.67 mg/m^2/day.

The calculations for other fluxes are similar:

At LC0, the mean %TOM =17.11%

In 100g of sediment, there is 17.11g TOM

In 11.11g sediment, TOM flux = 17.11gTOM/100g x 11.11 g/m^2/day = 1.90 g/m^2/day

At LC0, the mean %TC = 5.17%

In 100g of sediment, there is 5.17g TC

In 11.11g sediment, TC flux = 5.17gC/100g x 11.11 g/m^2/day = 0.57 g/m^2/day

The oxygen uptake rate is used as a measure of the organic matter utilization or decomposition by organisms. Smith (1978) had used the bottom sediment oxygen uptake, a measure of community

metabolism, as an indirect measure of organic carbon utilization. The oxygen uptake rate is used to calculate the rate of the carbon consumption.

At LC0, the mean oxygen uptake rate = 17.06 mmole/m^2/day

One mole of oxygen is equivalent to one mole of carbon consumed.

Hence for LC0, 17.06 mmole/m^2/day = 17.06 x 12 mgC/m^2/day consumed.

= 17.06 x 10^{-3} x 12g C/m^2/day consumed

= 0.20 gC/m^2/day

Hence the carbon consumed = 0.20 gC/m^2/day

Wassman (1984) found that the respiratory quotient of CO_2/O_2 is 0.85. In this work, the CO_2 emitted by sediments was not measured; hence the respiratory quotient was not determined. The assumption used here to calculate the carbon consumed is that the carbon source is carbohydrate.

The calculated carbon and organic matter budgets are given in Table 4.23. These values are 'effective fluxes' because they are calculated from the mean concentration of organic carbon preserved in the top 5cm of sediment and, therefore, do not include organic carbon which has been remineralized soon after deposition (Hedges and Mann, 1979b). All these fluxes are in the unit g/m^2/day. All the fluxes are estimates, as these are all based on the mean organic matter, carbon and lignin contents in the surface sediments and the oxygen consumption at the sediment-water interface. All the fluxes: labile organic matter, refractory organic matter, total organic matter, total carbon, and total lignin are higher in locations nearest the river source. Further down the loch, all the organic matter has already decreased, indicating the importance of the contribution of terrestrial organic matter into the loch. LC6 shows the lowest fluxes for all the organic matter. All these indicate the contribution of terrestrial organic matter from River Creran into the loch.

Table 4.23 Organic matter and carbon fluxes.

Locations	% labile OM	Flux of labile OM ($g/m^2/d$)	% refrac OM	Flux of refrac. OM ($g/m^2/d$)	%TOM	Flux of TOM ($g/m^2/d$)	%TC	Flux of TC ($g/m^2/d$)	Lignin (mg/g)	Lignin flux ($mg/m^2/d$)	Oxygen uptake ($mmole/m^2/d$)	Carbon consumed ($gC/m^2/d$)
LC0	9.83	1.09	7.28	0.81	17.11	1.90	5.17	0.57	0.3305	3.67	17.06	0.20
LC1	9.34	1.04	6.88	0.76	16.19	1.80	4.62	0.51	0.2180	2.42	20.78	0.25
LC2	6.61	0.73	6.15	0.68	12.76	1.42	3.67	0.41	0.1760	1.96	9.40	0.11
LC3	3.47	0.39	4.91	0.55	8.38	0.93	2.75	0.31	0.0682	0.76	12.44	0.15
LC5	3.43	0.38	3.09	0.34	6.63	0.74	3.19	0.35	0.1016	1.13	15.13	0.18
LC6	3.60	0.40	6.03	0.67	9.63	1.07	1.93	0.21	0.0470	0.52	9.44	0.11
Sediment trap	14.75	1.64	8.69	0.97	23.44	2.60	6.38	0.71	0.3041	3.38		0.25

OM = organic matter; TOM = total organic matter; TC = total carbon; refrac = refractory.

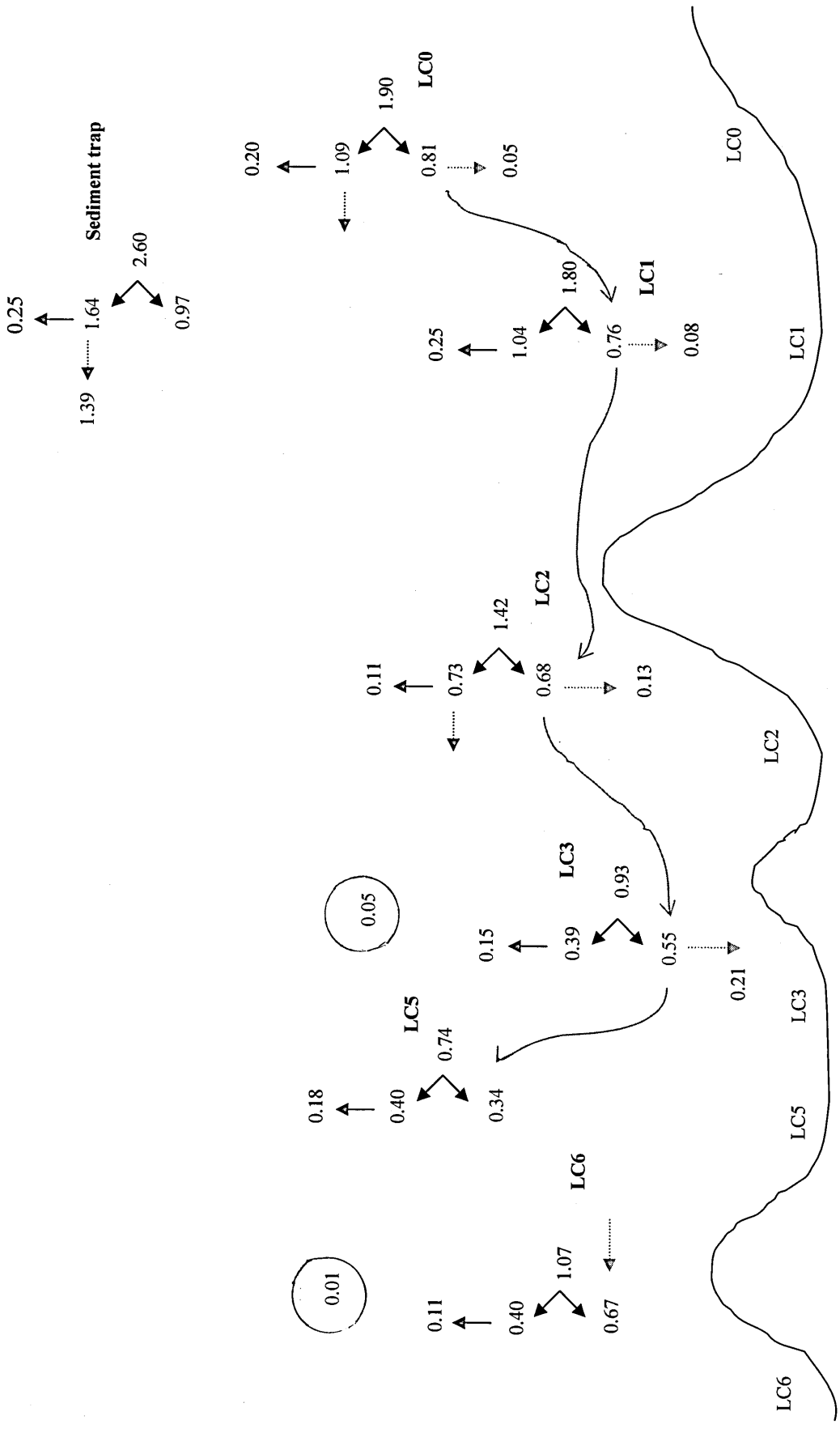
Sediment trap
0.25
1.64
1.39
2.60
0.97
0.20
1.09
1.90
LC0
0.81
0.05
0.25
1.04
1.80
LC1
0.76
0.08
0.11
0.73
1.42
LC2
0.68
0.13
0.05
0.15
0.39
0.93
LC3
0.55
0.21
0.18
0.40
LC5
0.74
0.34
0.01
0.11
0.40
1.07
LC6
0.67
LC0
LC1
LC2
LC3
LC5
LC6

Figure 4.17 Organic matter fluxes ($g/m^2/d$) in Loch Creran.

Keys:

Green arrows represent labile organic matter.

Brown arrows represent refractory organic matter.

Broken arrows represent remaining of the organic matter; brown broken arrows represent sedimentation of the materials; green broken arrows represent remain of the labile organic matter used for organic matter degradation.

Circles represent the remaining of the materials which were not known what happen to.

Figure 4.17 is firstly explained in detail for LC0. From the figure, the total flux of organic matter into the sediment at LC0 was 1.90 g/m^2/day. Of this, 1.09 g/m^2/day consists of the labile fraction of the organic matter while 0.81 g/m^2/day is of the % refractory organic matter. The 1.90 g/m^2/day organic matter also consists of 0.57 g/m^2/day TC and 3.67 mg/m^2/day lignin fluxes into the LC0 sediment. The shortcoming of this assumption is that it is not certain whether the TC and lignin constitute the % labile or % refractory fraction of the organic matter. As there is significant correlation between % labile organic matter with total lignin, and because lignin is the biomarker for terrestrial organic matter, these terrestrial materials are assumed to be components of the labile organic matter. The reason there is no significant correlation between lignin and the refractory fraction of the organic matter is because the refractory fraction of the organic matter most probably also consists of lignin material that has undergone diagenesis to unrecognizable structure. Significant correlation between lignin and the labile organic matter also confirms the ability of lignin to serve as biomarker for terrestrial organic matter (Section 4.4.3.2, Implications 1 and 2).

It is assumed that the carbon consumed, as calculated from the oxygen uptake rate, was mainly from the labile organic matter fraction. If 0.20 g/m^2/day carbon was consumed from the 1.09 g/m^2/day labile organic matter, there will be 0.89 g/m^2/day labile organic matter remains. The 3.67 mg/m^2/day lignin most probably contributes to this remaining fraction. Hence from the total organic matter input of 1.90 g/m^2/day into LC0, only 1.70 g/m^2/day (0.89 + 0.81 g/m^2/day) TOM remains. This will remain in the sediment, or transported to LC1 via near-bottom transport, or resuspended into the water column and transported down the loch via advective transport.

The basis for the following assumption is that the refractory fraction of the organic matter does not undergo much degradation hence most of this fraction remains in the water column and sediment. Of the 0.81 g/m^2/day refractory OM at LC0, it is assumed that 0.76 g/m^2/day was transported to LC1 because of the 0.76 g/m^2/day refractory organic matter is found at LC1. The remaining 0.05 g/m^2/day organic matter was assumed to have sedimented out. Of the 0.76

$g/m^2/day$ refractory OM reaching LC1, only the remaining 0.08 $g/m^2/day$ was deposited with the sediment as 0.68 $g/m^2/day$ refractory organic matter reached LC2. The continuation of the transport of materials this way occurred through resuspension of the sedimented particles, due to the entrainment and advective processes, and transport of these materials further down the loch with the outflowing water.

For the sediment trap samples, assuming the total carbon consumed is 0.25 $g/m^2/day$ (the same as the total carbon consumed at LC1 surface sediment) resulting in 1.39 $g/m^2/day$ labile organic matter remaining. The 1.39 $g/m^2/day$ along with the 0.97 $g/m^2/day$ refractory organic matter makes up 2.36 $g/m^2/day$ used organic matter. It is now known what happens to this organic matter. This 2.36 $g/m^2/day$ organic matter might be transported further down the loch, some are sedimented out and some undergo decomposition during transport.

Notice also that further down the loch at LC3, LC5 and LC6, the remaining labile organic matter decreased. This is not due to increased consumption of this organic matter, as seen from the almost steady oxygen uptake or carbon consumption rate. This is due to a reduction in the labile fraction of organic matter being transported further down the loch. Some possibilities include sedimentation, and lateral transport to sea, or release into the atmosphere as CO_2 (Grace and Malhi, 2002). As a result, the relative refractory fraction of the organic matter increases further down the loch.

Implications

The mean sedimentation rate 11.11g/m^2/d is obtained from the sediment trap situated near LC1. This mean value is then applied to calculate the fluxes along the Loch Creran. Some assumptions based on this usage are given.

1. One of the weaknesses in using 11.11g/m^2/d as the representative sedimentation rate for the whole loch is overestimation of the lignin, organic matter and carbon fluxes for locations further down the loch. This is because further down the loch, the mean sedimentation rate ought to be lower than 11.11g/m^2/d (equivalent to 240.9gC/m^2/yr; see Section 4.2.1.3, Implication 1 for the calculations). A good example is that Cronin and Tyler (1980) found the mean sedimentation rate at a location in the mid basin of Loch Creran was 50gC/m^2/yr, hence 11.11g/m^2/d (or 240.9gC/m^2/yr) at the uppermost basin is reasonable.

2. The effect of seasonal variation and materials re-suspension can be cancelled off. As 11.11g/m^2/d is the mean sedimentation rate for the duration of one year, this value does not account for the effect of seasonal variation. The effect of materials re-suspension at different stations is also cancelled as it is assumed that re-suspension of materials occurred at all stations along the loch.

3. As all the parameters decreased significantly from the head to the mouth of the loch, and the sedimentation rate is standardized, the effect of River Creran in contributing terrestrial materials into the loch becomes more distinct. The decrease of lignin, organic matter and carbon fluxes from the head to the mouth of Loch Creran (Table 4.22) confirms this.

4. Besides, only materials and experimental results from the sediment trap are used to calculate the carbon, lignin and organic matter fluxes per year (see Section 4.7.2). The

purpose of the calculation of fluxes at individual locations (Table 4.22 and Figure 4.17) provides only rough estimation for fluxes of materials at individual locations.

4.7.2 Organic matter and carbon fluxes on a global scale

In order to understand the global carbon cycle it is important to know how much organic carbon is buried in marine sediments, and in this way withdrawn from the global carbon cycle (De Hass and Van Weering, 1997). The preservation of organic matter is almost exclusively restricted to aquatic sediments (Welte, 1969) and this organic matter is derived from terrestrial, marine and anthropogenic sources. The total burial rate of organic carbon is ~0.16 x 10^{15} gC yr^{-1}, of which 90% is deposited in deltas and the continental shelves and upper slopes (Chester, 2000).

In order to calculate the carbon flux in g/year, the surface area of the uppermost basin of Loch Creran of 2.04km^2 (Section 1.3.2.1; Gage, 1972) is used because the sediment trap was situated near the outflowing end of this uppermost basin. LC1, the deepest basin in this uppermost basin, is also situated near the traps; hence all the materials in this uppermost basin are assumed to accumulate here. Example of the calculation is shown here:

For the sediment trap materials,

Organic matter flux = 2.60g/m^2/d x 2.04 x $(10^3)^2m^2$ x 1/[(1/365)yr] = 0.19 x 10^{10} g/yr

Labile organic matter flux = 1.64g/m^2/d x 2.04 x $(10^3)^2m^2$ x 1/[(1/365)yr] = 0.12 x 10^{10} g/yr

Refractory organic matter flux = 0.97g/m^2/d x 2.04 x $(10^3)^2m^2$ x 1/[(1/365)yr] = 0.07 x 10^{10} g/yr

Total carbon (TC) flux = 0.71g/m^2/d x 2.04 x $(10^3)^2m^2$ x 1/[(1/365)yr] = 0.05 x 10^{10} g/yr

Lignin flux = 3.38 mg/m^2/d x 2.04 x $(10^3)^2m^2$ x 1/[(1/365)yr] = 0.25 x 10^7 mg/yr

Carbon consumed = 0.25g/m^2/d x 2.04 x $(10^3)^2m^2$ x 1/[(1/365)yr] = 0.02 x 10^{10} g/yr

From the above results, the input of organic matter into Loch Creran is estimated to be 0.19x10^{10}g/yr. Of this 0.12x10^{10}g/yr (or 63.16%) was the labile fraction, and 0.07x10^{10}g/yr

(36.84%) was the refractory fraction. The input of total carbon into Loch Creran was $0.05x10^{10}$gC/yr. The total lignin input from the River Creran was $0.25x10^{7}$g/yr and total carbon consumed was $0.02x10^{10}$g/yr.

Globally, 35% (81×10^{12} g C yr^{-1}) of the labile fraction may become oxidized in estuaries and in the marine environment. The rest (150×10^{12} g C yr^{-1}) appears to be highly degraded, with the bulk entering present-day tropical and subtropical sea areas. This degraded fraction could represent a significant source of organic carbon accumulating in marine sediments (Ittekkot, 1988). De Haas and Van Weering (1997) found that the organic carbon accumulation rate in the Norwegian Channel was 0.17×10^{6} tons/yr (equivalent to 0.17×10^{9} g/yr), and 0.83×10^{6} tons/yr (equivalent to 0.83×10^{9} g/yr) in the Skagerrak. Lobbes *et al.* (2000) found that the 12 Russian rivers studied transported about 10×10^{12} g of total organic carbon per year into the Arctic Ocean, and the total lignin discharge was about 42×10^{9} g/yr.

In Loch Creran, the percentage labile and refractory fractions of the organic matter were estimated to be 63.16% and 36.84%, respectively. It seems that the percentage labile organic matter input into the loch was more than the refractory fraction. The reason being the estimation made in this study was carried out at the uppermost basin near the head of the river input, hence there was still labile organic matter remaining in this location.

Lobbes *et al.* (2000) estimated about 10×10^{12} gTOC per year input into the Arctic Ocean from 12 Russian rivers. The average TOC input from one river would be 8.33×10^{11} g/yr. Hence the total carbon flux into Loch Creran (0.5×10^{9} g/yr) is more than the Norwegian channel (0.17×10^{9} g/yr) and less than the Skagerrak (0.83×10^{9} g/yr), and less than the TOC input from the Russian rivers.

CHAPTER 5 CONCLUSION AND FUTURE WORK

5.1 CONCLUSION

From this research, the ability of the lignin oxidation products to serve as biomarkers for terrestrial organic matter was demonstrated. As they are produced only by vascular plant tissues, the detection of lignin in the sediments provides a straightforward method for tracing the input and output of terrestrial organic matter along the lochs Creran and Etive. Overall the total lignin concentration in both lochs ranged from 0.33 to 0.55mg/g, and the Λ ranged from 0.37 to 0.98. The (Ad/Al)v values in these two lochs ranged from 0 to 7.61, the S/V ratios ranged from 0.16 to 1.61, and the C/V ratios ranged from 0 to 1.47. Two important factors determine the lignin concentrations in the sediments within a studied system: the distance from the freshwater or river input, and the catchment area draining into the system. It was found that the lignin content Λ (S+V+C mg/100mgOC) in both lochs Creran and Etive was within the values reported from other locations from previous studies. These values are lower than the Λ values obtained from some other locations studied because of the smaller catchment area of the lochs compared to larger catchment area such as the Amazon. The lower Λ values detected in some other locations is because these locations were situated further from the river sources. As most of the coastal and marine areas studied are open systems, there are constant input and output of materials within these systems, hence the worldwide Λ values are within the range 0.06-6.8. The mean (Ad/Al)v value for lochs Creran and Etive was however, slightly higher than the mean (Ad/Al)v values obtained from past studies. This was attributed mostly to water circulation and ventilation in Loch Creran, degradation of lignin materials prior to the input into the lochs, and the type of vegetation which was non-woody angiosperms, in the lochs.

Lignin oxidation products are also able to distinguish between different plant sources, as different plant types produce different lignin phenol groups: angiosperms produce syringyl (S) and vanillyl

(V) phenols, gymnosperms produce only vanillyl phenols, non-woody tissues produce cinnamyl (C) phenols, and woody tissues do not produce cinnamyl phenols. Hence the ratio of syringyl to vanillyl (S/V) and the cinnamyl to vanillyl phenols (C/V) ratios were used to determine the vegetation source of the sediments in the lochs. From the lignin compositional plots of S/V versus C/V, S versus C, S versus V, and C versus V, it was found that non-woody angiosperm tissues predominate in the two Scottish sea lochs. Both lochs have deciduous trees adjacent to the shore, whereas spruce is usually found on higher grounds, hence the detection of the non-woody angiosperm tissues in the sediments of the lochs. This is also confirmed using the aerial photographs of the vegetation surrounding Loch Creran and via personal observation. As lignin decreased from the head to the mouth of the lochs, contribution of terrestrial materials mostly originated from the catchment of Rivers Creran and Etive. Besides being used to determine the vegetation source, the S, V and C phenols could also be used to indicate diagenesis. In Loch Creran, the percentage decrease of the lignin phenol groups from LC0 to LC5 are as follows: V (68.04%), S (74.92%) and C (65.63%). In Loch Etive, the percentages decrease of the lignin phenol groups from RE2 to RE6 are as follow: V (6.38%), S (14.18%) and C (29.42%). Higher percentage degradation in sediments from Loch Creran is due to the hydrodynamic sorting process, which was not prominent in Loch Etive. Besides, Loch Creran has more dynamic circulation of the water system and more active ventilation than Etive. Hence sediments from Loch Creran were more degraded, whereas sediments from Loch Etive were still susceptible to degradation. This is also evidenced from the slightly higher (Ad/Al)v and Rp values in Loch Creran than Etive. The mean (Ad/Al)v and Rp values from LC1 to LC5 are 0.91 and 0.50, respectively. In Loch Etive, the mean (Ad/Al)v and Rp values from RE2 to RE6 are 0.82 and 0.42, respectively.

At individual sampling locations, the lignin, organic matter and carbon contents all showed constancy throughout the year. There was no distinct seasonal trend in the decrease and increase of the organic matter concentration. For example, during the few winter months, there was no increase in the lignin, organic matter and carbon contents at individual locations. There was only

slight increase in the carbon and organic matter contents during the summer in the sediment trap samples, and this was also evidenced by the slight increase in $\delta^{13}C$ values, all indicating the contribution of marine organic matter. It was also found that the increased in rainfall during February and June 2002 resulted in increase sedimentation rates in April and August 2002. There were significant increases (ANOVA: $p<0.05$) in the % labile and % total organic matter in the sediment trap samples in February, March and June 2002. At LC1, there was an increase in % labile organic matter during March and August 2002. Sediments from LC1 also showed significant increase in %TC for April 2002. Lignin content increased significantly for the sediment trap samples in March and July 2002, and at LC1 during March and June 2002. Hence although there was no significant correlation between the environmental parameters with lignin, organic matter and carbon content, the increase of these organic materials in months following heavy rainfall and high sedimentation rate indicate the effect of seasonal variation on the organic matter content, albeit slightly. Secondly, the constant lignin, organic matter and carbon content at individual locations also indicate an intermittent but continual input of terrestrial materials from the river sources into the lochs, and constant transport of these materials out of the lochs. There was however, no fixed time for these episodic inputs.

The effect of seasonal variation on the carbon content at individual locations was not as distinct as the carbon distribution along transect of the lochs. The accumulation of materials near the head of the lochs, and the gradual transport of these materials further down the loch, result in decreased organic matter content from the head to the mouth of the lochs. This phenomenon also stressed the importance of rivers in contributing terrestrial materials into the lochs. The lignin, organic matter, carbon and phosphate contents decreased from the head to the mouth of the lochs. Significant correlation between the $\delta^{13}C$ values and total lignin confirmed the importance of rivers as the source of terrestrial materials. Mean total lignin decreased from 0.33mg/g at LC0 to 0.05mg/g at LC6. Percentage organic matter due to loss on ignition decreased from 23.44% in the sediment trap sample to 6.53% at LC5. The mean $\delta^{13}C$ values increased from -25.21‰ at LC0 to -14.30‰ at LC6. The mean %TC decreased from 6.38% for the sediment trap samples to

1.92% at LC6. Total phosphate decreased from 0.109μg/g at LC1 to 0.025μg/g at LC6, and total inorganic phosphate decreased from 0.99μg/g at LC1 to 0.015μg/g at LC6. In Loch Etive, mean total lignin decreased from 0.55mg/g at RE2 to 0.07mg/g at Camas Nathais. Percentage total organic matter decreased from 22.80% at RE6 to 15.11% at Camas Nathais. The $\delta^{13}C$ values decreased from increased from -25.71‰ at RE2 to -11.25‰ at Camas Nathais. Mean %TC decreased from 6.25% at RE2 to 3.49% at Camas Nathais. Besides, the importance of the contribution from the rivers, the significant decrease of organic matter from the head to the mouth of the lochs, also signify the sedimentation of materials during transport, and dilution of terrestrial materials with marine organic matter further down the lochs. The importance of rivers in contributing terrestrial materials into the loch, was also confirmed by the detection of lignin in the dissolved and particulate fraction from the water sample collected from River Creran. The decrease of the oxygen uptake rates from the head to mouth of Loch Creran indicates that these terrestrial materials affect the biodegradability of the sediment organic matter. Overall the oxygen uptake rates in Loch Creran decreased from 20.44mmole/m^2/d to 9.44mmole/m^2/d from LC1 to LC6.

In this research, biodegradability of the sediment organic matter was successfully measured using several proxies. Overall the Rp index was found to be the best proxy to determine the biodegradability of the sediment organic matter. First of all, the Rp index is the ratio of the refractory to total organic matter hence it is a direct measure of the lability of the sediment organic matter. Also, the Rp index has significant (simple regression analysis: $p<0.05$) positive correlation with other parameters such as the $\delta^{13}C$ values, %TC, %TN, and total lignin. The Rp index is obtained from the loss on ignition experiment, a simple, economic and easy analytical method to use. The next best proxy would be the oxygen uptake rate, as this is a direct measure of sediment biodegradability. Higher oxygen uptake rate indicates higher rate of organic matter decomposition or higher degradability of sediment organic matter, and *vice versa*. The C/N ratio is also a direct indicator of the degradation stage of organic matter, and used along with the Rp

index, is a more powerful tool. Significant correlation of these proxies with total lignin indicates the importance of terrestrial organic matter in fuelling the biogeochemical cycling in the lochs.

The mean sedimentation rate in Loch Creran was 11.11g/m^2/d. As this is the mean value for the sedimentation rates for 11 months, the effect of seasonal variation is cancelled. However the impact of material resuspension on this value is unknown as the sedimentation rates were not monitored for a series of depth profiles. The use of 11.11g/m^2/d as the mean sedimentation rate is also based on findings from past studies that the 10m below surface water as the most reliable depth to represent the average sedimentation rate throughout the water column. The mean sedimentation rate of 11.11g/m^2/d obtained in this study for Loch Creran is higher compared to the rate obtained by Cronin and Tyler (1980), most probably because the sediment traps were situated in the uppermost basin of the loch near the input of terrestrial materials. Also the traps were located near the Creagan narrows hence the effect of water renewal is strong here. The high sedimentation rate results in fresher materials trapped in the bottom sediment, resulting in higher (Ad/Al)v and Rp values in the surface sediments at times, as fast deposition moves organic matter down with less total degradation.

There were several transport mechanisms in the lochs: vertical transport of materials onto the surface sediments, advective transport due to entrainment of particles with the incoming denser saline water following by transport of particles with the out flowing water, and gravity current transport due to differences in the depths. Loch Creran is the smaller of the two lochs hence there is no modification of the tidal cycle here compared to Loch Etive. Exchange and mixing with seawater are rapid in Loch Creran, and there was sufficient flushing to ensure good ventilation of the bottom water, as a result there was no stagnation of water in this loch. The entrainment of particles transports the less dense non-woody tissues out of the loch with the out flowing water. The coarser and denser plant materials consisting of woody tissues are retained in the inner shelf. This process is known as hydrodynamic sorting, a mechanism which plays an important role transporting terrestrial materials from the head to the mouth of Loch Creran. This phenomenon is also evidenced by the increase of the C/V ratios, indicating the increase of non-

woody tissues further down the loch. In Loch Etive, the large input of freshwater into the loch, which is relatively long, a situation where there was occasional stagnation of water was created, resulting in two distinct layers: the less dense freshwater at the upper layer, and the denser saline water at the bottom. During this period, there was no entrainment process to sort out the non-woody and woody tissues hence the hydrodynamic sorting phenomenon is less in Loch Etive. This was evidenced by the decrease in the C/V ratios further down the loch, most probably due to the input of new materials. The catchment to surface area ratio for Loch Etive is 49.49, and for Loch Creran 12.12. Hence the lignin, organic matter and carbon inventories for Loch Etive were higher than Loch Creran.

In Loch Creran, lignin accounted for only 0.69% TOC at LC1, and this decreased to 0.43% at LC5 and 0.47% at LC6. In Loch Etive, lignin contributed approximately 0.91% TOC at RE2, decreasing to 0.33% at Camas Nathais. The higher contribution of lignin to %TOC in Loch Etive is most probably due to the higher catchment to loch area ratio. In Loch Creran, the terrestrial organic matter contributed 78% of total organic matter at LC0, and this decreased to 52% at LC5, and 11% at LC6. The contribution of marine organic matter increased in the reverse order: 22% at LC0 and 89% at LC6. In Loch Etive, terrestrial organic matter contributed 84-85% of total organic matter within the loch, and 15-16% for Camas Nathais. Here marine organic matter contributed in reverse order: 15-16% within the loch, and 84-85% at Camas Nathais. Further evidence that lignin is more highly resistant to biodegradation compared to other terrestrial organic matter is the increase of the % lignin contribution to terrestrial organic matter from the head to the mouth of the lochs. At LC0, the percentage contribution of lignin to terrestrial organic matter was 0.25%, and this increases to 0.50% at LC6. The percentage contributions of lignin to terrestrial organic matter in locations in Loch Etive were: RE2 (0.34%), RE5 (0.25%), RE6 (0.24%) and Camas Nathais (0.35%). As the more labile fraction of organic matter was degraded and due to the constancy of lignin materials in the environments, the ratio of lignin to total terrestrial organic matter increased gradually down the lochs.

Lignin flux, as well as organic matter and TC fluxes in Loch Creran, decreased from the head to the mouth and outside the loch. Lignin flux at LC0 was 3.67mg/m^2/day, and this decreased to 1.13 mg/m^2/day at LC5. The % labile organic matter flux at LC0 was 1.09g/m^2/day, and this decreased to 0.38g/m^2/day at LC5; the % refractory organic matter flux at LC0 was 0.81g/m^2/day and this decreased to 0.34g/m^2/day at LC6; the % total organic matter flux at LC0 was 1.90g/m^2/day and this decreased to 0.74g/m^2/day at LC6; the total carbon flux into LC0 was 0.57g/m^2/day, decreasing to 0.35g/m^2/day at LC5. All these indicate sedimentation out during transport and utilization of organic matter by organisms.

The organic matter input into Loch Creran is estimated to be 0.19×10^{10}g/yr. Of this 0.12×10^{10}g/yr (or 63.16%) was the labile fraction, and 0.07×10^{10}g/yr (36.84%) was the refractory fraction. The input of total carbon into Loch Creran was 0.05×10^{10}gC/yr. The total lignin input from the River Creran was 2.5×10^{6}g/yr and total organic matter buried was estimated to be 0.02×10^{10}g/yr.

In summary, these findings will be added to the scientific literature upon the successful completion of this work. The first two findings have also been reported by previous studies. Some new findings from this piece of work are given following these.

- The importance of lignin as the biomarker for terrestrial organic matter.
- The importance of the contribution of terrestrial organic matter into the coastal marine environments.
- Experimentally, thorough investigation of the CuO oxidation method has made improvement on several procedural steps. The method validation experiment also confirmed the good reproducibility of the loss on ignition method.
- The lignin content, Λ, in lochs Creran and Etive was compared to values from locations worldwide. It was found that the Λ values worldwide were within a definite range between 0.06 and 6.8.
- Besides being caused by high rates of organic matter degradation and dynamic water circulation, the slightly higher (Ad/Al)v values found in lochs Creran and Etive might

also be caused by the type of vegetation throughout the catchment, the non-woody angiosperm tissues.

- Terrestrial organic matter plays an important role in fuelling the biogeochemical cycling in sea lochs. Hence, in sea lochs these terrestrial materials govern the biodegradability of sediment organic matter.
- The biodegradability of sediment organic matter was successfully measured using these proxies: Rp index, oxygen uptake rate and the C/N ratio.
- Combination of these parameters explains what happens to terrestrial organic matter after being transported into the aquatic environments, and what happens to these materials during subsequent transportation.
- All parameters were also used to calculate the lignin, carbon and organic matter budgets in a Scottish sea loch, Loch Creran.

Overall the success of this work lies in the importance of the unique characteristic of lignin. One is the low biodegradability of the lignin materials, hence the lignin distribution is related to transport down the lochs. As they are produced only by vascular land plants, detection of lignin in the sediments indicates the presence of terrestrial materials in the lochs. Due to the relative resistance of lignin to biodegradation in the aquatic, and especially in the anaerobic environments, these materials accumulate to a greater extent in the sediments in relative to other substrates such as carbohydrates and proteins. Hence, although lignins contribute only a small amount to the total carbon and bulk terrestrial materials, lignin can be detected. Secondly, lignin materials produce simple lignin-derived phenols upon oxidation with CuO and each lignin-derived phenol is representative of a vegetation type. As a result, these lignin phenols are used to determine the vegetation source of terrestrial organic matter in the lochs.

The surface sediments in both lochs Creran and Etive inner basin sediments are not anoxic, and there should be lignin degradation occurring. However, the (Ad/Al)v values in the lochs did not

show significant change from the head to the mouth of the lochs. These small changes in the (Ad/Al)v values also means that the lignin materials in both lochs undergo lignin side-chain and ring structure degradation at similar rates through the reaction of aquatic bacteria. Besides the high and constant (Ad/Al)v values throughout the lochs means that these lignin materials underwent degradation prior to entering the lochs.

Naturally, lignin is relatively resistant to biodegradation. Ultimately lignin does degrade albeit very slowly. As the lignin materials had mostly undergone degradation prior to entering the loch, they would undergo further degradation more slowly in the loch. In Loch Creran, bioturbation seems to play an important role in assisting organic matter degradation within the surface 0 to 10cm sediment layer. Periodic exposure to oxygen due to activities of burrowing animals results in enhanced degradation of materials deeper down the sediments.

5.2 STRENGTHS AND WEAKNESSES OF THIS STUDY

- In this research, the detailed method validation experiments were carried out in order to obtain optimisation of the analytical methods. The lignin analysis, loss on ignition, CN analysis and carbon isotope analyses were carried out for some known compounds. The purpose of these analyses was to confirm the validity of each experimental method, which they did. These validation analyses are one of few experiments carried out to date for the respective experimental methods. Known plant samples were subjected to the lignin analysis, carbon isotope determination and the CN analysis; these analyses were also one of the few such experiments carried out to date.
- Water samples were collected from the River Creran in order to quantify lignin in the dissolved and particulate fractions. Lignin phenols were successfully elucidated from the suspended particulate fraction of water samples.
- Terrestrial organic matter does play an important role in fuelling the biogeochemical cycling in the two Scottish sea lochs, lochs Creran and Etive. Several parameters were

successfully used as the proxies to determine the biodegradability of the sediment organic matter: oxygen uptake rate, Rp index, C/N ratio and inorganic phosphate content.

- The differences in organic matter quality and quantity between Loch Creran and Loch Etive are due to the differences in the hydrographical and hydrodynamic regimes of the lochs.
- Several weaknesses found in this study are also given here. The River Creran flow rate was measured using a very basic method. Although lignin was successfully determined from water this was only from one sample. The input of the dissolved and particulate lignin fraction was not successfully determined due to the limited number of samples collected. These analyses provided only a preliminary determination. More validation experiments, and sample collection should have been undertaken.

  The phosphate results could not be used to assess seasonal variation. As it was found that all the other experimental parameters were very constant at individual locations, the original idea was to analyse several samples in random order in order to obtain the mean phosphate concentration at individual locations, so that the phosphate content along transect of the lochs could be compared with other parameters. However, it now seems that more analyses should have been undertaken in order to determine the seasonal variation of the phosphate content at individual locations. Also, method validation for this analysis is needed as the percentage reproducibility showed relatively high variation for the few months studied.

5.3 THE FUTURE

- Phosphate analysis definitely warrants further investigation, as the inorganic phosphate content appears to be correlated significantly (regression: $p<0.05$) with several experimental parameters, whilst the oxygen uptake rate only correlated with the inorganic phosphate content. The inorganic phosphate might therefore be the most

sensitive proxy for the biodegradability of organic matter. Unfortunately, the very few analyses reported here cannot provide this confirmation.

- Determination of the dissolved and particulate components of the lignin fraction, as well as total organic matter, would be essential in refining the estimation of the lignin, carbon and organic matter budgets.
- Experimentally, the microwave digestion method as used by Goni and Montgomery (2000), should be given priority, as this method is easy to use, time-saving and uses lower volume of solvent.
- Previous authors (Hedges and Mann, 1979b; Hedges *et al.*, 1982 and 1985; Ishiwatari and Uzaki, 1987; and Kastner and Goni, 2003) had determined the depth profile for sediments from several geographic regions and found that these regions contain stable lignin materials for several hundred to million years. These authors had analyzed sediment cores from 10cm to 700m depth. It would be interesting to know the depth profile of lignin materials in Lochs Creran and Etive, in order to determine the vegetation changes and hence, the climatic change in this part of the world since a few hundreds to thousands of years ago.

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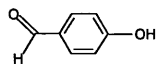
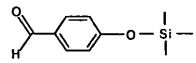
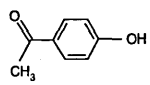
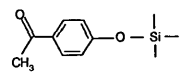
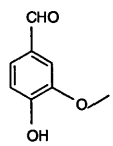
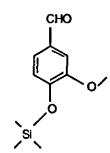
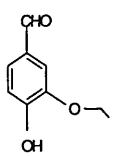
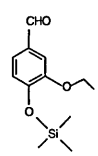
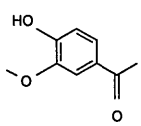
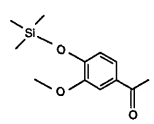
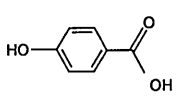
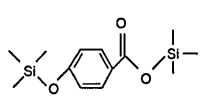
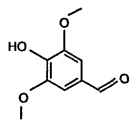
APPENDICES

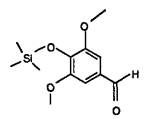
Appendix 1 Lignin-derived phenols, their trimethylsilated products and formula weight.

Lignin monomer	Silylated components of lignin monomer
p-Hydroxybenzaldehyde	
	
FW 122.12	FW 194.21
p-Hydroxyacetophenone	
	
FW 136.15	FW 208.24
Vanillin (4-hydroxy-3-methoxybenzaldehyde)	
	
FW 152.15	FW 224.24
Ethyl vanillin (3-ethoxy-4-hydroxybenzaldehyde)	
	
FW 166.18	FW 238.27
Acetovanillone (3-methoxy-4-hydroxyacetophenone)	
	
FW 166.18	FW 238.27
p-Hydroxybenzoic acid	
	
FW 138.12	FW 282.3

Syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde)

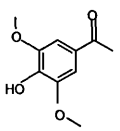


FW 182.18

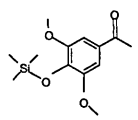


FW 254.27

Acetosyringone (3,5-dimethoxy-4-hydroxy-acetophenone)

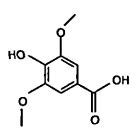


FW 196.2

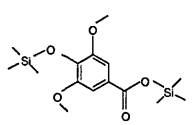


FW 268.29

Syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid)

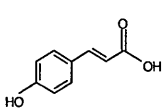


FW 198.17

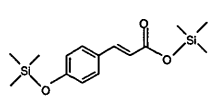


FW 342.35

p-Coumaric acid (p-hydroxycinnamic acid)

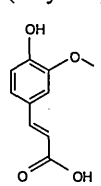


FW 164.16

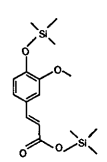


FW 308.34

Ferulic acid (4-hydroxy-3-methoxycinnamic acid)

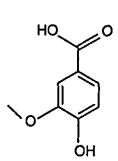


FW 194.19

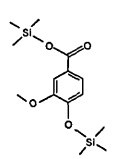


FW 338.37

Vanillic acid (benzoic acid, 4-hydroxy-3-methoxy-)



FW 168.15



FW 312.33

The trimethylsilated products are the products produced by CuO oxidation and separated by the gas chromatography.

Appendix 2 An example of a chromatogram for sediment sample from LC1. This is an actual chromatogram obtained from the GC-FID, scanned and presented as follows.

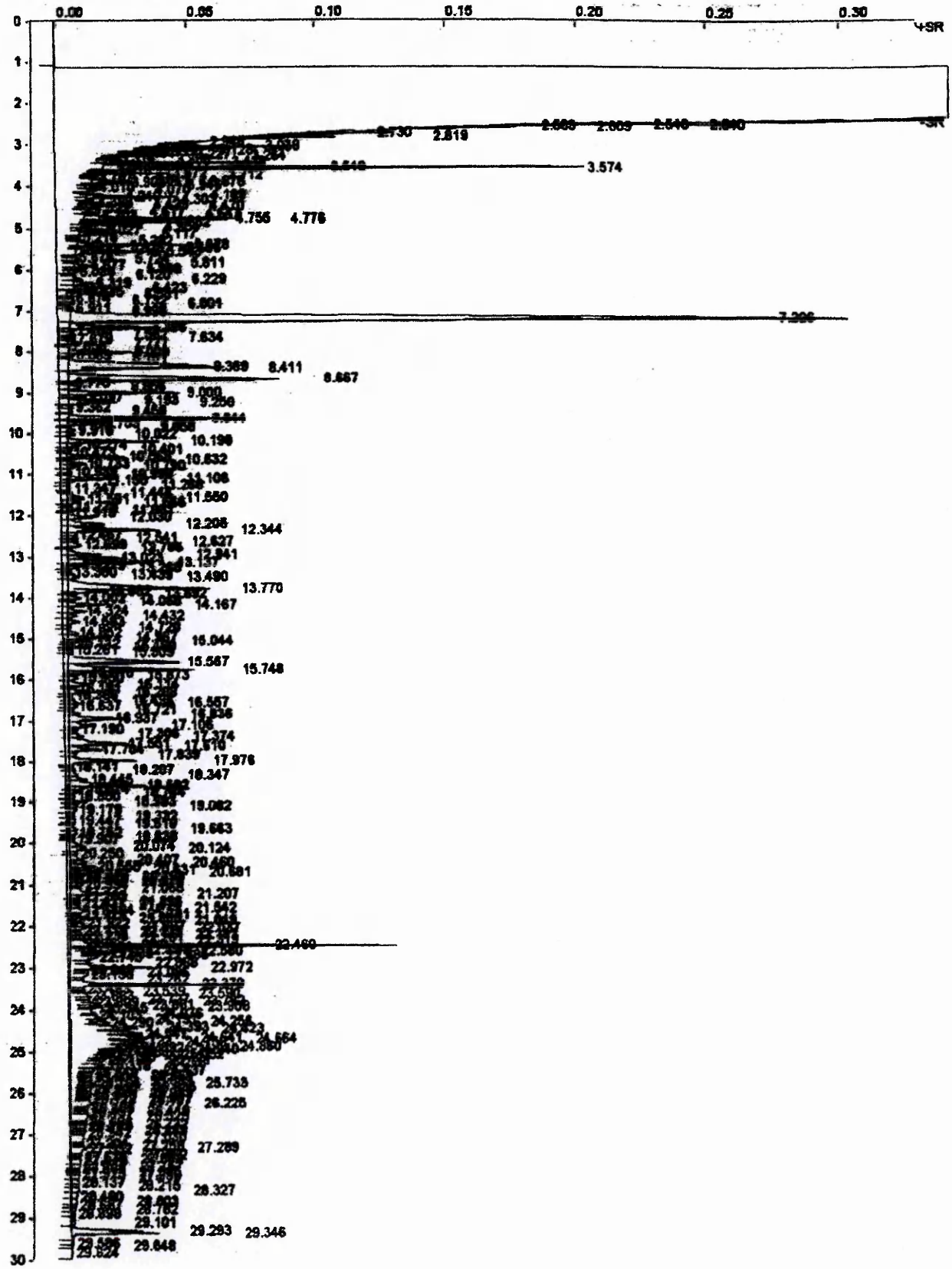
njection Date: 23-JUN-3 6:47 PM Calculation Date: 23-JUN-3 7:17 PM

perator :
Workstation:
nstrument : Varian Star #1
hannel : B = B

Detector Type: ADCB (10 Volts)
Bus Address : 16
Sample Rate : 10.00 Hz
Run Time : 30.002 min

********** Star Chromatography Workstation ******* Version 4.5 ***************

hart Speed = 0.75 cm/min Attenuation = 143 Zero Offset = 2%
tart Time = 0.000 min End Time = 30.000 min Min / Tick = 1.00



Title

Run Mode : Analysis
Peak Measurement: Peak Area
Calculation Type: Percent

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)	Status Codes
1		0.3978	2.540	0.000	4969	TS	0.0	
2		0.1331	2.583	0.000	1663	TS	0.0	
3		0.1117	2.609	0.000	1395	TS	0.0	
4		0.3392	2.730	0.000	4237	TS	0.0	
5		0.2580	2.819	0.000	3223	TS	0.0	
6		0.0185	2.946	0.000	232	TS	0.0	
7		0.4724	3.039	0.000	5900	TS	0.0	
8		0.1871	3.093	0.000	2338	TS	0.0	
9		0.2134	3.128	0.000	2666	TS	0.0	
10		0.0083	3.181	0.000	104	TF	0.0	
11		0.3618	3.227	0.000	4519	TF	0.0	
12		0.0195	3.264	0.000	244	TS	0.0	
13		0.0287	3.314	0.000	359	TS	0.0	
14		0.0256	3.356	0.000	320	TS	0.0	
15		0.5926	3.416	0.000	7402	TS	0.0	
16		2.8677	3.549	0.000	35819	TF	0.0	
17		0.0758	3.574	0.000	947	TF	0.0	
18		0.0556	3.616	0.000	694	TS	0.0	
19		0.2299	3.672	0.000	2872	TS	0.0	
20		0.0143	3.712	0.000	179	TS	0.0	
21		0.0225	3.767	0.000	281	TS	0.0	
22		0.0701	3.841	0.000	876	TF	0.0	
23		0.1714	3.876	0.000	2141	TF	0.0	
24		0.1708	3.905	0.000	2133	TF	0.0	
25		0.0467	3.946	0.000	583	TF	0.0	
26		0.0579	4.010	0.000	723	TF	0.0	
27		0.0791	4.070	0.000	989	TF	0.0	
28		0.0742	4.189	0.000	927	TF	0.0	
29		0.4529	4.240	0.000	5657	TF	0.0	
30		0.0622	4.303	0.000	777	TF	0.0	
31		0.0716	4.386	0.000	895	TF	0.0	
32		0.0958	4.429	0.000	1197	TF	0.0	
33		0.0834	4.470	0.000	1042	TF	0.0	
34		0.0573	4.523	0.000	715	TF	0.0	
35		0.0934	4.617	0.000	1167	TF	0.0	
36		0.0595	4.647	0.000	743	TF	0.0	
37		0.0801	4.703	0.000	1000	TF	0.0	
38		0.8726	4.755	0.000	10899	TF	0.0	
39		0.6196	4.776	0.000	7740	TF	0.0	
40	1) 4-hb.	0.5149	4.832	0.000	✓6432	TF	0.0	
41		0.2367	4.881	0.000	2957	TF	0.0	
42		0.0638	4.954	0.000	797	TF	0.0	
43		0.2032	5.027	0.000	2538	TF	0.0	
44		0.1898	5.117	0.000	2370	TF	0.0	
45		0.0066	5.216	0.000	83	TS	0.0	
46		0.0356	5.282	0.000	444	TF	0.0	
47		0.6646	5.378	0.000	8302	TF	0.0	
48		0.0410	5.424	0.000	512	TF	0.0	
49		0.0186	5.456	0.000	233	TF	0.0	
50		0.0334	5.488	0.000	418	TF	0.0	
51		0.6358	5.543	0.000	7942	TF	0.0	
52		0.0067	5.676	0.000	84	TF	0.0	
53		0.0461	5.724	0.000	576	TF	0.0	
54		0.0282	5.811	0.000	352	TF	0.0	
55		0.1791	5.877	0.000	2237	TF	0.0	
56		0.0757	5.968	0.000	945	TF	0.0	
57		0.0296	6.035	0.000	370	TF	0.0	
58		0.0106	6.120	0.000	133	TF	0.0	

59		0.0970	6.229	0.000	1211	TF	0.0	
60		0.2024	6.319	0.000	2528	TF	0.0	
61	2) 4-hd	0.3198	6.423	0.000	3994	TF	0.0	
62		0.2286	6.535	0.000	2856	TF	0.0	
63		0.0782	6.581	0.000	977	TF	0.0	
64		0.0043	6.674	0.000	54	TF	0.0	
65		0.0267	6.732	0.000	334	TF	0.0	
66		0.0301	6.801	0.000	375	TF	0.0	
67		0.0271	6.911	0.000	339	TF	0.0	
68		0.0429	6.996	0.000	536	TF	0.0	
69	*	15.2963	7.226	0.000	191060	TF	0.0	
70		0.2171	7.325	0.000	2711	TF	0.0	
71	3) vanillin.	0.7778	7.396	0.000	9715	TF	0.0	

59		0.0970	6.229	0.000	1211	TF	0.0
60		0.2024	6.319	0.000	2528	TF	0.0
61	2) 4-hb	0.3198	6.423	0.000	3994	TF	0.0
62		0.2286	6.535	0.000	2856	TF	0.0
63		0.0782	6.581	0.000	977	TF	0.0
64		0.0043	6.674	0.000	54	TF	0.0
65		0.0267	6.732	0.000	334	TF	0.0
66		0.0301	6.801	0.000	375	TF	0.0
67		0.0271	6.911	0.000	339	TF	0.0
68		0.0429	6.996	0.000	536	TF	0.0
69	*	15.2963	7.226	0.000	191060	TF	0.0
70		0.2171	7.325	0.000	2711	TF	0.0
71	3) vanillin	0.7778	7.396	0.000	9715	TF	0.0
72		0.0303	7.463	0.000	379	TF	0.0
73		0.0518	7.562	0.000	647	TF	0.0
74		0.0520	7.634	0.000	650	TF	0.0
75		0.0365	7.679	0.000	455	TF	0.0
76		0.1115	7.777	0.000	1393	TF	0.0
77		0.0012	7.858	0.000	15	TS	0.0
78		0.6092	8.009	0.000	7609	TF	0.0
79		0.0578	8.089	0.000	722	TF	0.0
80		0.0277	8.151	0.000	346	TF	0.0
81		3.0104	8.369	0.000	37601	TF	0.0
82		0.3119	8.411	0.000	3896	TF	0.0
83	4) EV	3.2622	8.667	0.000	40747	TF	0.0
84		0.0422	8.770	0.000	528	TF	0.0
85		0.1933	8.856	0.000	2414	TF	0.0
86	5) acetovan	1.0071	9.000	0.000	12579	TF	0.0
87		0.1469	9.097	0.000	1835	TF	0.0
88		0.2844	9.193	0.000	3552	TF	0.0
89		0.4004	9.250	0.000	5001	TF	0.0
90		0.0412	9.362	0.000	515	TF	0.0
91		0.0537	9.458	0.000	671	TF	0.0
92	6) 4-hba	1.6622	9.644	0.000	20762	TS	0.0
93		0.3354	9.753	0.000	4189	TF	0.0
94		0.0181	9.858	0.000	226	TF	0.0
95		0.0722	9.916	0.000	902	TF	0.0
96		0.0654	10.022	0.000	817	TF	0.0
97		0.7612	10.198	0.000	9508	TF	0.0
98		0.1592	10.274	0.000	1988	TF	0.0
99		0.0342	10.401	0.000	427	TF	0.0
100		0.0204	10.473	0.000	255	TF	0.0
101	7) syringal	0.5223	10.565	0.000	6524	TF	0.0
102		0.0210	10.632	0.000	262	TF	0.0
103		0.1526	10.733	0.000	1906	TF	0.0
104		0.1061	10.790	0.000	1325	TF	0.0
105		0.0451	10.939	0.000	563	TF	0.0
106		0.0340	10.998	0.000	425	TF	0.0
107		0.4012	11.106	0.000	5012	TF	0.0
108		0.0630	11.155	0.000	787	TF	0.0
109		0.0177	11.266	0.000	221	TF	0.0
110		0.0333	11.347	0.000	416	TF	0.0
111		0.0395	11.444	0.000	493	TF	0.0
112		0.1653	11.550	0.000	2065	TF	0.0
113		0.1294	11.591	0.000	1616	TF	0.0
114		0.1158	11.666	0.000	1446	TF	0.0
115		0.0421	11.778	0.000	526	TF	0.0
116		0.0799	11.851	0.000	999	TF	0.0
117		0.0139	11.918	0.000	174	TF	0.0
118		0.3157	12.030	0.000	3944	TF	0.0
119		0.5524	12.205	0.000	6900	TF	0.0
120		1.0039	12.344	0.000	12539	TF	0.0
121	8) acetosyr	0.1053	12.467	0.000	1315	TF	0.0
122		0.1181	12.541	0.000	1475	TF	0.0
123		0.1018	12.627	0.000	1271	TF	0.0
124	9) pl	0.1390	12.699	0.000	1736	TF	0.0
125		0.0147	12.785	0.000	184	TF	0.0
126		0.3581	12.941	0.000	4473	TF	0.0
127		0.4933	13.023	0.000	6161	TF	0.0
128		0.7862	13.137	0.000	9820	TF	0.0
129		0.1422	13.219	0.000	1776	TF	0.0
130		0.0698	13.268	0.000	872	TF	0.0
131		0.0276	13.380	0.000	344	TF	0.0
132		0.0533	13.439	0.000	665	TF	0.0
133		0.1070	13.490	0.000	1336	TF	0.0
134		2.2315	13.770	0.000	27872	TF	0.0
135		0.3587	13.832	0.000	4481	TF	0.0
136		0.0636	13.892	0.000	794	TF	0.0
137		0.1404	14.002	0.000	1753	TF	0.0
138		0.0491	14.068	0.000	614	TF	0.0

117	0.0139	11.918	0.000	174	TF	0.0
118	0.3157	12.030	0.000	3944	TF	0.0
119	0.5524	12.205	0.000	6900	TF	0.0
120	1.0039	12.344	0.000	12539	TF	0.0
121	0.1053	12.467	0.000	1315	TF	0.0
122	0.1181	12.541	0.000	1475	TF	0.0
123	0.1018	12.627	0.000	1271	TF	0.0
124	0.1390	12.699	0.000	1736	TF	0.0
125	0.0147	12.785	0.000	184	TF	0.0
126	0.3581	12.941	0.000	4473	TF	0.0
127	0.4933	13.023	0.000	6161	TF	0.0
128	0.7862	13.137	0.000	9820	TF	0.0
129	0.1422	13.219	0.000	1776	TF	0.0
130	0.0698	13.268	0.000	872	TF	0.0
131	0.0276	13.380	0.000	344	TF	0.0
132	0.0533	13.439	0.000	665	TF	0.0
133	0.1070	13.490	0.000	1336	TF	0.0
134	2.2315	13.770	0.000	27872	TF	0.0
135	0.3587	13.832	0.000	4481	TF	0.0
136	0.0636	13.892	0.000	794	TF	0.0
137	0.1404	14.002	0.000	1753	TF	0.0
138	0.0491	14.068	0.000	614	TF	0.0

8) acetopyr

9) gl

139	0.3995	14.167	0.000	4990	TF	0.0
140	0.1888	14.324	0.000	2358	TF	0.0
141	0.0901	14.432	0.000	1125	TF	0.0
142	0.1451	14.583	0.000	1813	TF	0.0
143	0.0234	14.720	0.000	293	TF	0.0
144	0.1013	14.852	0.000	1265	TF	0.0
145	0.4130	14.967	0.000	5159	TF	0.0
146	0.1494	15.044	0.000	1866	TF	0.0
147	0.0560	15.112	0.000	699	TF	0.0
148	0.0625	15.189	0.000	780	TF	0.0
149	0.0293	15.261	0.000	366	TF	0.0
150	0.0449	15.309	0.000	561	TF	0.0
151	1.8821	15.567	0.000	23508	TF	0.0
152	1.5514	15.748	0.000	19378	TF	0.0
153	0.1914	15.816	0.000	2390	TF	0.0
154	0.0737	15.873	0.000	920	TF	0.0
155	0.1208	15.950	0.000	1509	TF	0.0
156	0.2652	16.114	0.000	3312	TF	0.0
157	0.0599	16.191	0.000	748	TF	0.0
158	0.2896	16.288	0.000	3618	TF	0.0
159	0.0279	16.366	0.000	349	TF	0.0
160	0.0649	16.496	0.000	810	TF	0.0
161	0.0952	16.557	0.000	1190	TF	0.0
162	0.1009	16.637	0.000	1260	TF	0.0
163	0.0329	16.721	0.000	411	TF	0.0
164	0.1405	16.836	0.000	1755	TF	0.0
165	0.6592	16.937	0.000	8234	TF	0.0
166	0.3776	17.106	0.000	4716	TF	0.0
167	0.0911	17.190	0.000	1137	TF	0.0
168	0.1154	17.306	0.000	1441	TF	0.0
169	0.1520	17.374	0.000	1899	TF	0.0
170	0.8889	17.551	0.000	11103	TF	0.0
171	0.0501	17.610	0.000	626	TF	0.0
172	0.4233	17.704	0.000	5288	TF	0.0
173	0.0995	17.839	0.000	1242	TF	0.0
174	0.7474	17.976	0.000	9336	TF	0.0
175	0.0232	18.141	0.000	289	TF	0.0
176	0.0731	18.207	0.000	913	TF	0.0
177	0.1517	18.347	0.000	1895	TF	0.0
178	0.2293	18.445	0.000	2864	TF	0.0
179	0.9965	18.592	0.000	12446	TF	0.0
180	0.1750	18.674	0.000	2185	TF	0.0
181	0.0936	18.744	0.000	1169	TF	0.0
182	0.0703	18.850	0.000	878	TF	0.0
183	0.1683	18.983	0.000	2102	TF	0.0
184	0.1212	19.082	0.000	1514	TF	0.0
185	0.1127	19.179	0.000	1408	TF	0.0
186	0.0640	19.332	0.000	799	TF	0.0
187	0.0857	19.447	0.000	1070	TF	0.0
188	0.0392	19.519	0.000	489	TF	0.0
189	0.1088	19.663	0.000	1360	TF	0.0
190	0.0785	19.752	0.000	981	TF	0.0

10) VA

11) p-cou

12) fe

Appendix 3 Results for the oxygen uptake tests.

(a) LC1.

Date	Core	Rep		Mean	Stdev	CV	Mean (3)	Stdev	CV
4.4.2002	1	1	17.26	17.40	0.14	0.80	14.50	2.99	20.62
		2	17.41						
		3	17.53						
	2	1	11.66	11.42	0.30	2.63			
		2	11.51						
		3	11.08						
	3	1	14.99	14.68	0.30	2.04			
		2	14.39						
		3	14.67						
7.5.2002	1	1	27.42	27.91	0.43	1.54	21.20	6.08	28.68
		2	28.20						
		3	28.11						
	2	1	19.70	19.61	0.08	0.41			
		2	19.55						
		3	19.58						
	3	1	15.81	16.07	0.25	1.56			
		2	16.10						
		3	16.30						
5.6.2002	1	1	18.42	18.92	0.60	3.17	18.67	3.28	17.57
		2	18.75						
		3	19.59						
	2	1	21.64	21.81	0.19	0.87			
		2	22.01						
		3	21.79						
	3	1	14.67	15.27	0.58	3.80			
		2	15.83						
		3	15.32						
2.7.2002	1	1	29.73	29.60	0.12	0.41	26.36	3.84	14.57
		2	29.50						
		3	29.57						
	2	1	27.30	27.36	0.09	0.33			
		2	27.42						
		3							
	3	1	22.18	22.12	0.16	0.72			
		2	21.94						
		3	22.24						
1.8.2002	1	1	24.75	24.34	0.35	1.44	27.78	3.19	11.48
		2	24.17						
		3	24.12						
	2	1	28.37	28.36	0.21	0.74			
		2	28.15						
		3	28.57						
	3	1	30.33	30.64	0.45	1.47			
		2	31.15						
		3	30.43						

(a) LC1 continued.

Date	Core	Rep		Mean	Stdev	CV	Mean (3)	Stdev	CV
2.9.2002	1	1	29.19	29.31	0.13	0.44	23.52	5.27	22.40
		2	29.30						
		3	29.44						
	2	1	19.26	19.01	0.51	2.68			
		2	19.35						
		3	18.42						
	3	1	22.30	22.25	0.06	0.27			
		2	22.28						
		3	22.18						
30.9.2002	1	1	21.89	21.76	0.13	0.60	18.87	4.10	21.73
		2	21.63						
		3	21.78						
	2	1	16.08	15.98	0.10	0.63			
		2	15.94						
		3	15.90						
14.11.2002	1	1	27.01	27.04	0.14	0.52	24.31	2.41	9.91
		2	27.18						
		3	26.91						
	2	1	22.42	22.50	0.31	1.38			
		2	22.24						
		3	22.83						
	3	1	23.42	23.39	0.04	0.17			
		2	23.39						
		3	23.35						
12.12.2002	1	1	10.96	11.25	0.25	2.22	18.70	8.34	44.60
		2	11.39						
		3	11.39						
	2	1	17.11	17.15	0.08	0.47			
		2	17.10						
		3	17.24						
	3	1	28.72	27.71	0.91	3.28			
		2	27.44						
		3	26.98						

(b) LC0.

Date	Core	Rep		Mean	Stdev	CV	Mean (3)	Stdev	CV
4.4.2002	1	1	19.12	19.13	0.04	0.21	18.65	1.60	8.58
		2	19.09						
		3	19.18						
	2	1	19.77	19.96	0.21	1.05			
		2	20.18						
		3	19.93						
	3	1	16.92	16.87	0.06	0.36			
		2	16.89						
		3	16.80						
4.6.2002	1	1	17.45	17.23	0.20	1.16	15.55	2.38	15.31
		2	17.05						
		3	17.18						
	2	1	13.97	13.86	0.30	2.16			
		2	14.09						
		3	13.51						
14.11.2002	1	1	19.94	19.95	0.04	0.20	15.87	3.88	24.45
		2	19.99						
		3	19.91						
	2	1	11.94	12.22	0.44	3.60			
		2	12.73						
		3	11.99						
	3	1	14.94	15.44	0.48	3.11			
		2	15.89						
		3	15.49						

(c) LC2.

Date	Core	Rep		Mean	Stdev	CV	Mean (3)	Stdev	CV
4.4.2002	1	1	9.20	9.13	0.09	0.99	9.23	0.17	1.84
		2	9.02						
		3	9.15						
	2	1	9.60	9.43	0.36	3.82			
		2	9.66						
		3	9.01						
	3	1	9.03	9.14	0.26	2.84			
		2	8.95						
		3	9.44						
2.9.2002	1	1	13.81	13.86	0.05	0.36	9.57	3.72	38.87
		2	13.87						
		3	13.90						
	2	1	7.14	7.34	0.58	7.90			
		2	6.89						
		3	8.00						
	3	1	7.51	7.51	0.04	0.53			
		2	7.55						
		3	7.46						

(d) LC3.

Date	Core	Rep		Mean	Stdev	CV	Mean (3)	Stdev	CV
21.3.2002	1	1	10.04	9.95	0.08	0.80	8.95	1.02	11.40
		2	9.91						
		3	9.90						
	2	1	7.92	7.91	0.02	0.25			
		2	7.89						
		3	7.92						
	3	1	9.18	8.99	0.17	1.89			
		2	8.96						
		3	8.84						
7.5.2002	1	1	13.08	13.60	0.64	4.71	13.58	1.86	13.70
		2	13.40						
		3	14.31						
	2	1	15.85	15.43	0.40	2.59			
		2	15.38						
		3	15.07						
	3	1	11.88	11.71	0.23	1.96			
		2	11.81						
		3	11.45						
30.9.2002	1	1	12.30	12.95	0.57	4.40	14.78	2.59	17.52
		2	13.34						
		3	13.22						
	2	1	16.31	16.61	0.30	1.81			
		2	16.62						
		3	16.91						

(e) LC5.

Date	Core	Rep		Mean	Stdev	CV	Mean (3)	Stdev	CV
21.3.2002	1	1	16.15	16.18	0.03	0.19	12.32	3.36	27.27
		2	16.17						
		3	16.21						
	2	1	10.82	10.83	0.03	0.28			
		2	10.86						
		3	10.80						
	3	1	9.84	9.96	0.11	1.10			
		2	10.01						
		3	10.04						
2.7.2002	1	1	15.43	15.52	0.10	0.64	14.36	2.51	17.48
		2	15.62						
		3	15.51						
	2	1	11.70	11.48	0.21	1.83			
		2	11.28						
		3	11.47						
	3	1	15.68	16.08	0.36	2.24			
		2	16.39						
		3	16.17						
12.12.2002	1	1	9.69	10.17	0.69	6.78	11.84	1.57	13.26
		2	10.66						
	2	1	12.34	12.06	0.36	2.99			
		2	12.20						
		3	11.65						
	3	1	12.87	13.28	0.54	4.07			
		2	13.89						
		3	13.08						

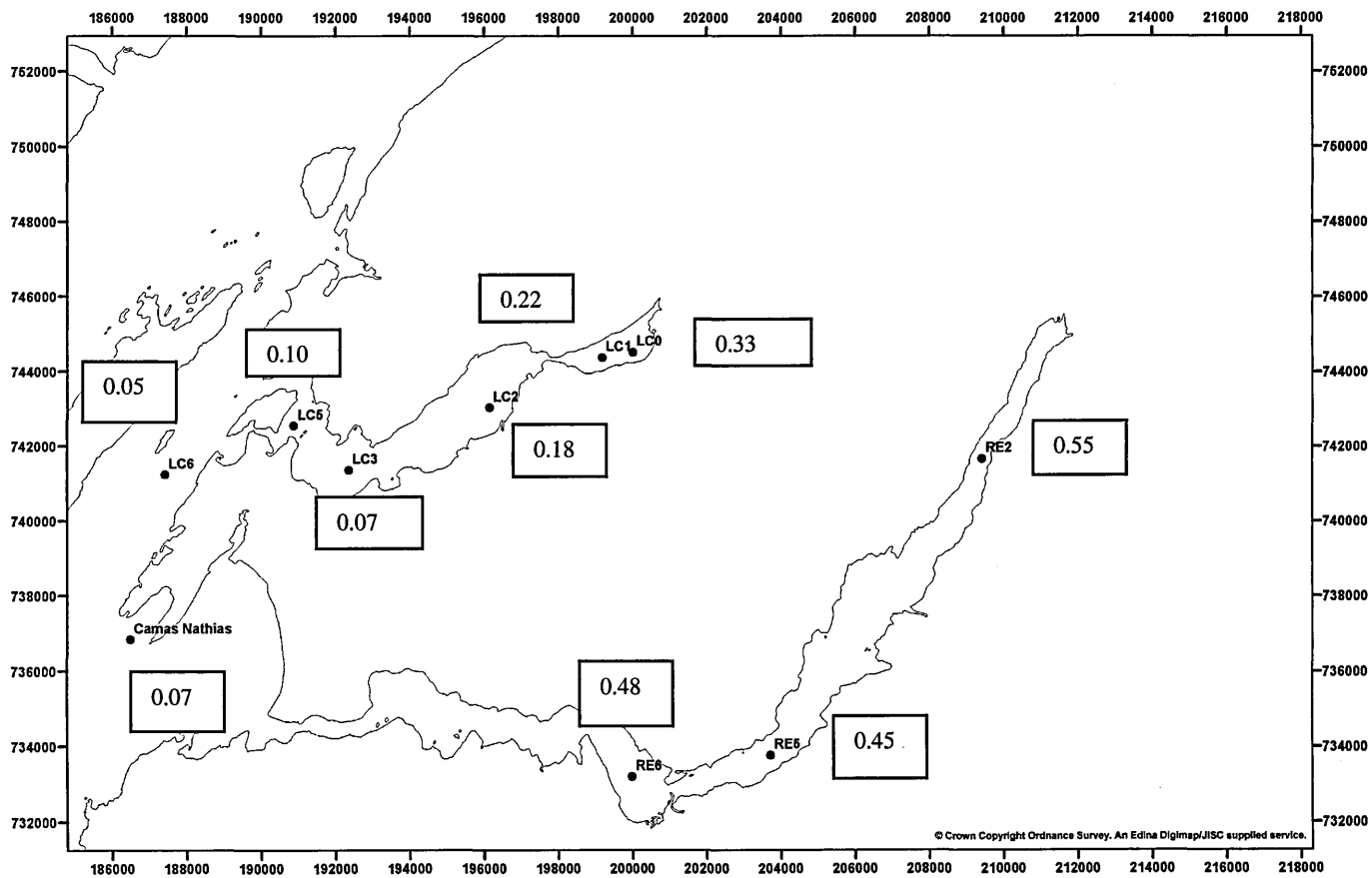
(f) LC6.

Date	Core	Rep		Mean	Stdev	CV	Mean (3)	Stdev	CV
21.3.2002	1	1	6.24	6.32	0.17	2.69	6.63	0.49	7.39
		2	6.22						
		3	6.51						
	2	1	6.52	6.37	0.24	3.77			
		2	6.50						
		3	6.10						
	3	1	7.27	7.20	0.06	0.83			
		2	7.18						
		3	7.15						
1.8.2002	1	1	10.89	10.74	0.38	3.54	14.18	3.16	22.28
		2	10.31						
		3	11.02						
	2	1	17.00	16.97	0.07	0.41			
		2	16.89						
		3	17.01						
	3	1	14.93	14.83	0.12	0.81			
		2	14.87						
		3	14.69						
30.9.2002	1	1	9.60	9.59	0.01	0.10	7.50	2.97	39.60
		2	9.58						
		3	9.58						
	2	1	5.29	5.40	0.13	2.41			
		2	5.36						
		3	5.50						

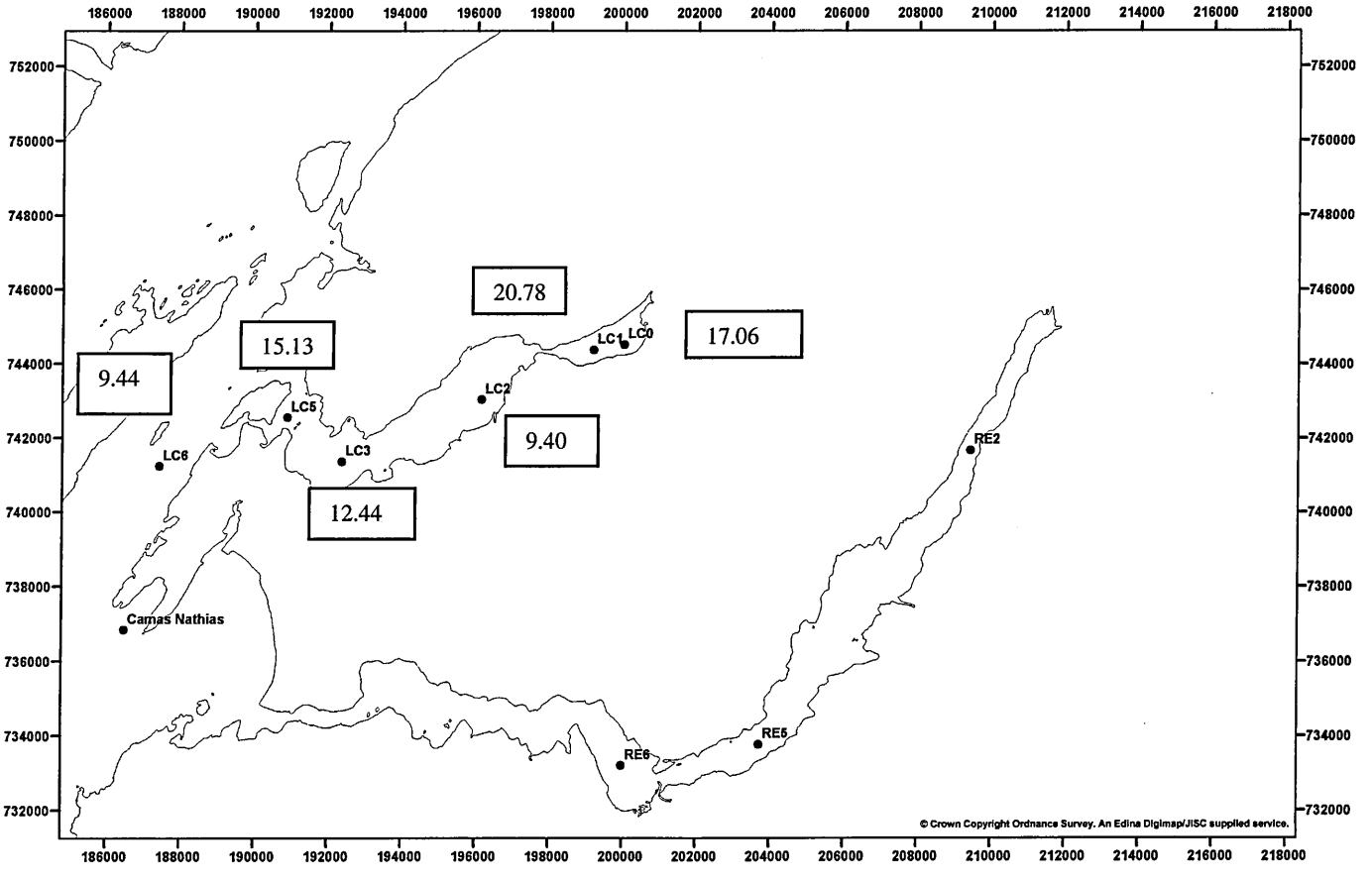
Three sediment cores were analysed for the oxygen uptake rate each time. For each core, there were triplicate analyses, that is, there were three syringes of water samples collected. Hence, there were three means for three different cores, respectively. And the final mean, labeled here as mean (3), is obtained for these three means. "CV" is the coefficient of variation. Rep = replicates.

Appendix 4 Distribution maps for all the experimental parameters.

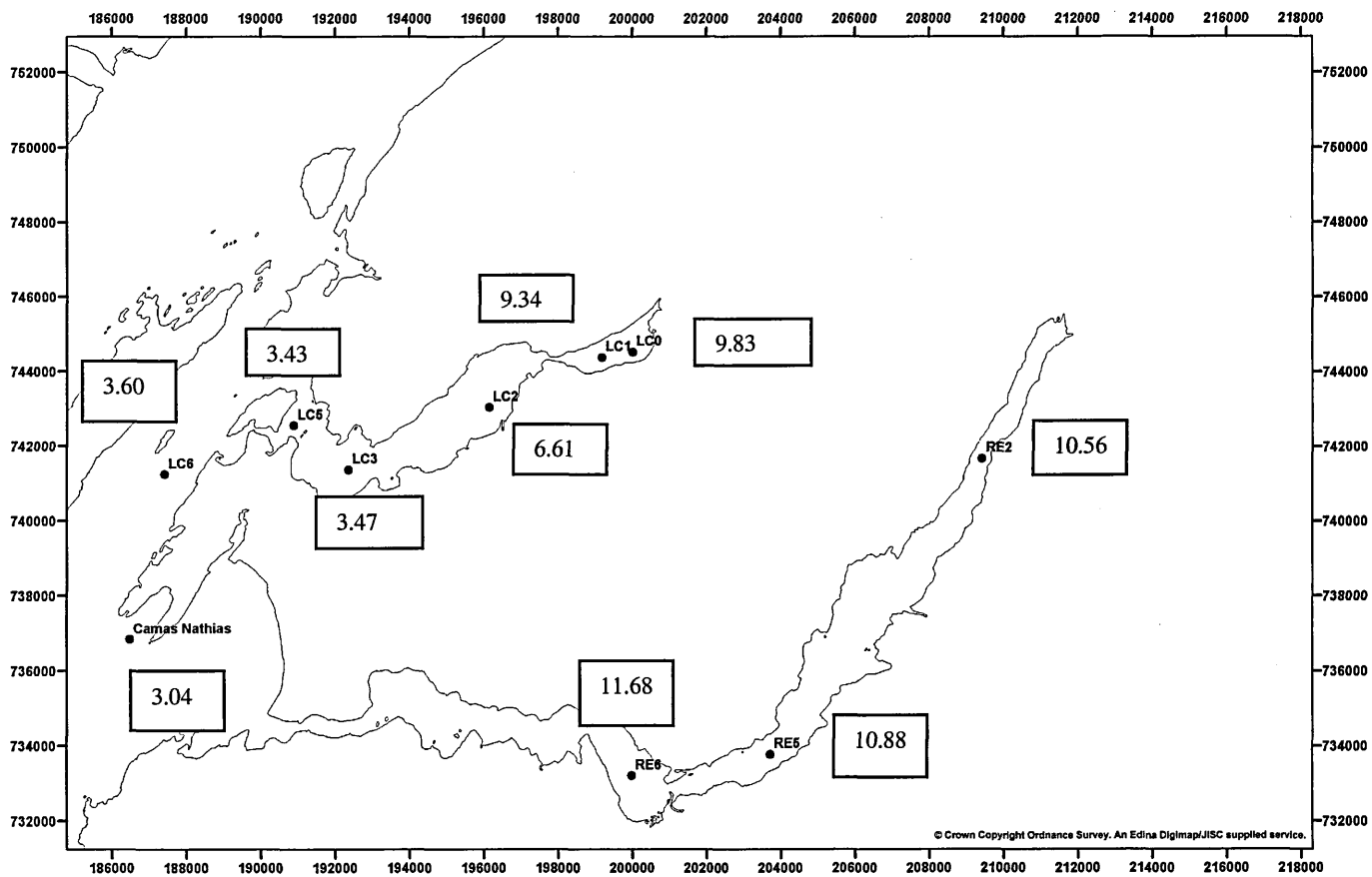
(a) Total lignin (mg/g).



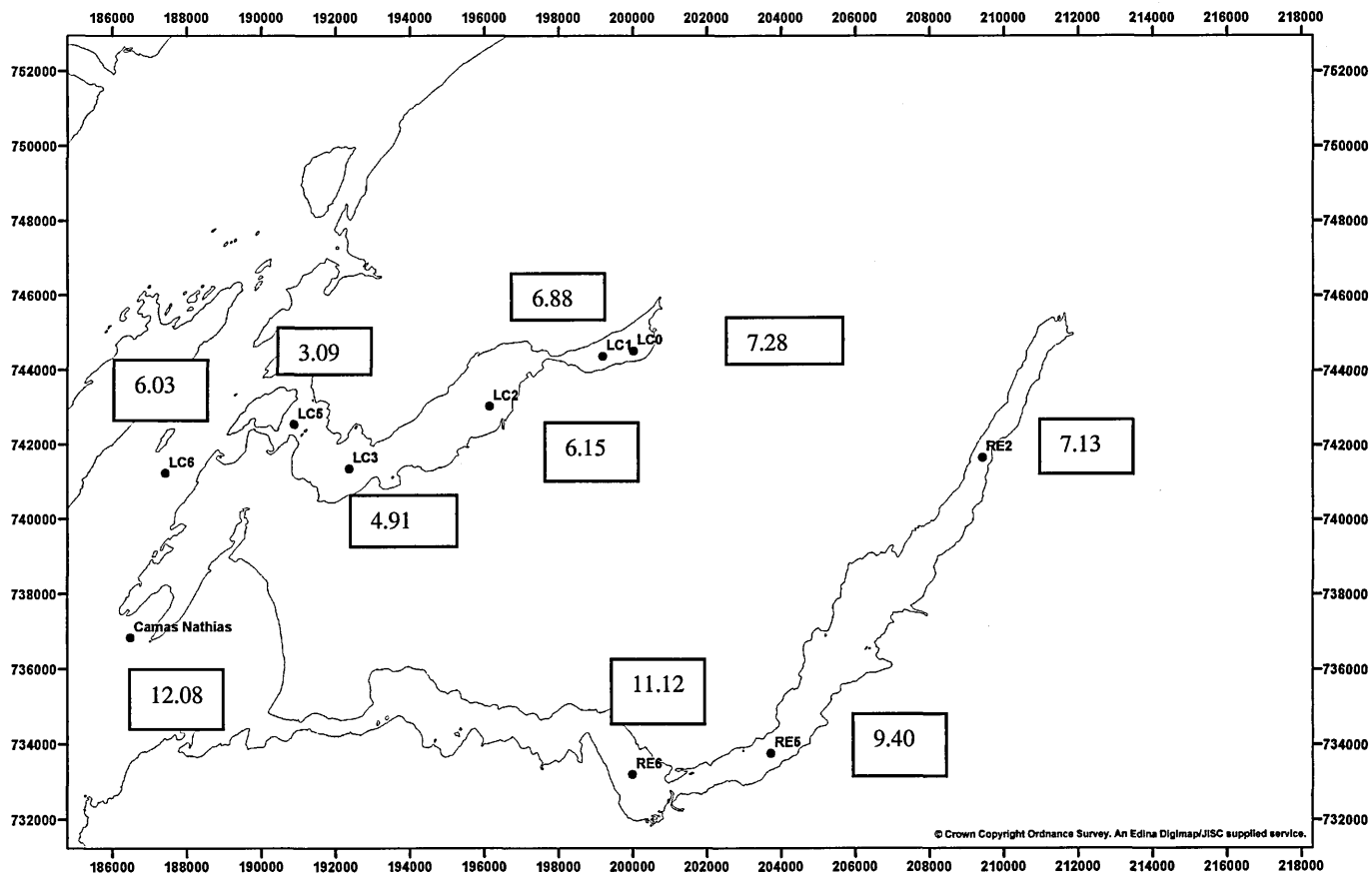
(b) O_2 uptake rate (mmole/m^2/day), only in Loch Creran.



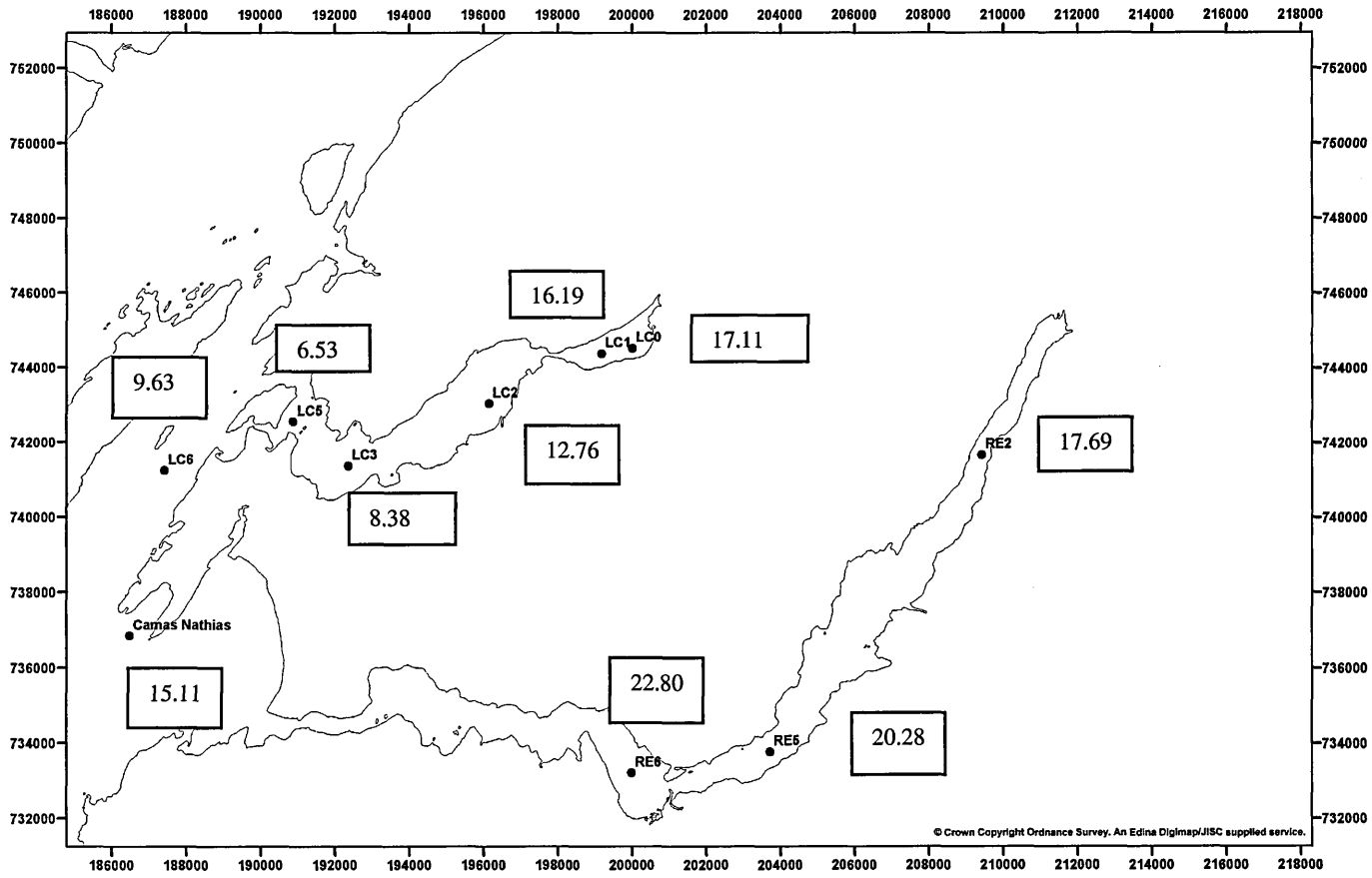
(c) Percentage labile organic matter due to loss on ignition (%).



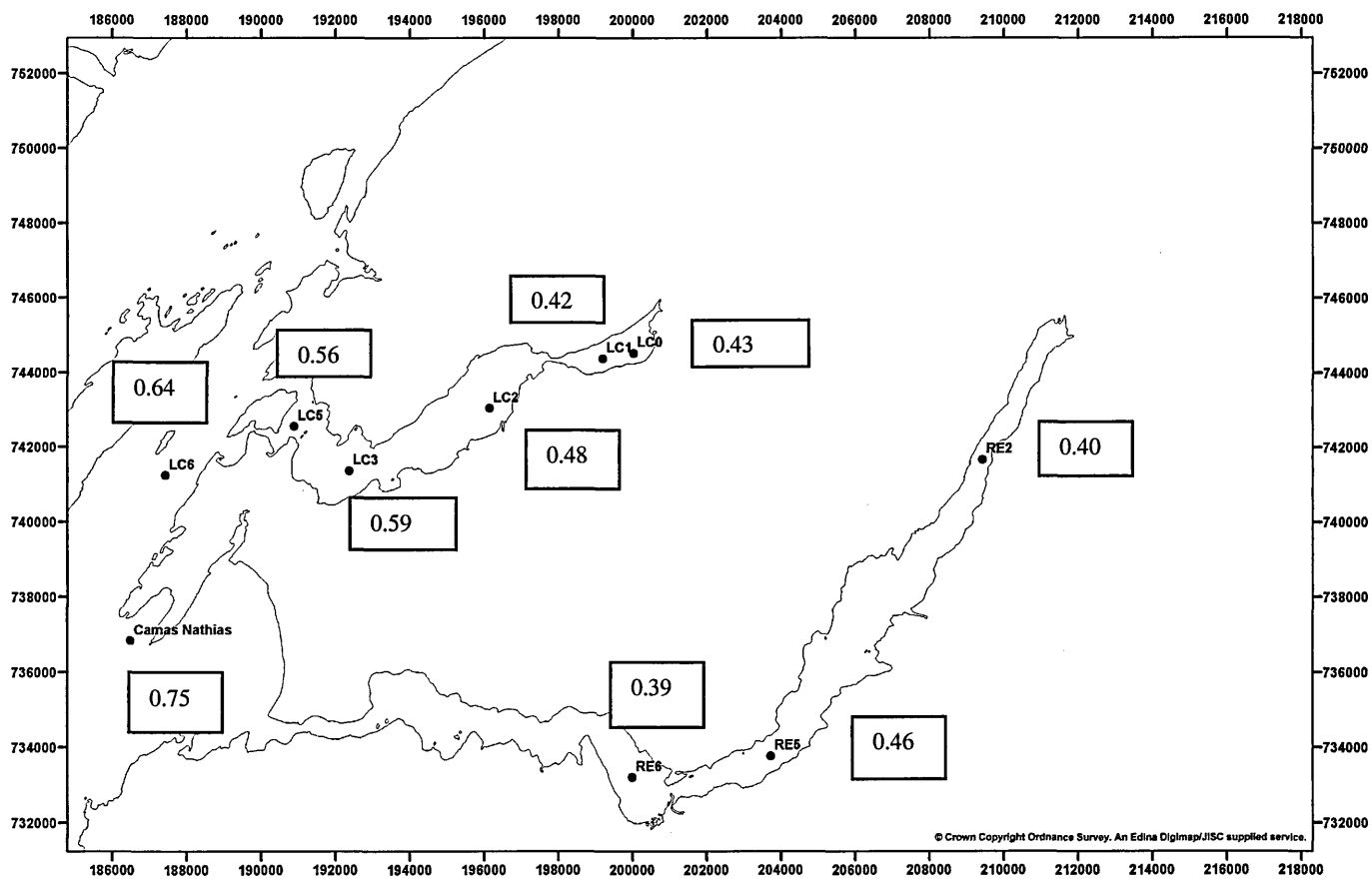
(d) Percentage refractory organic matter due to loss on ignition (%).



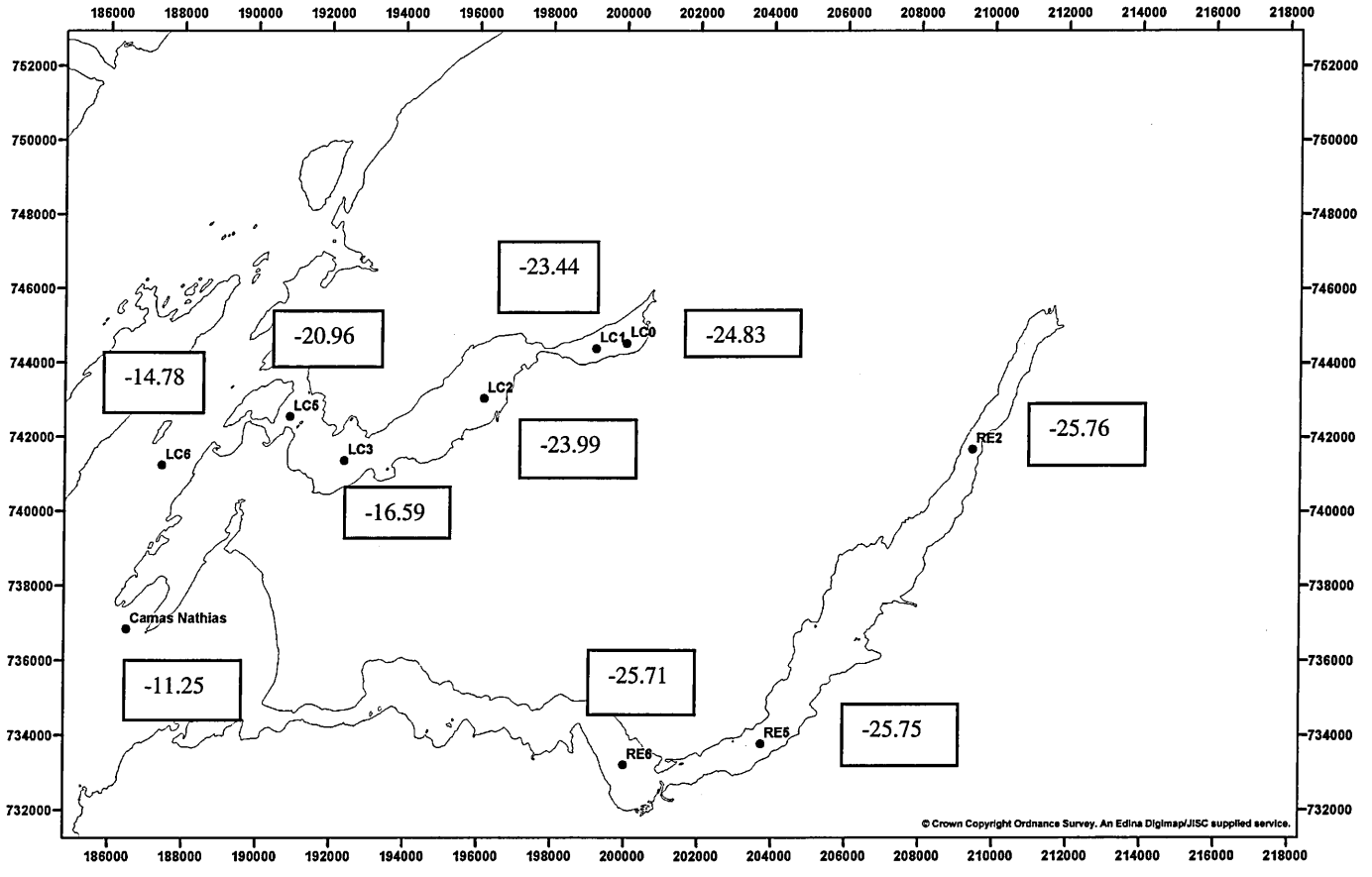
(e) Percentage total organic matter due to loss on ignition (%).



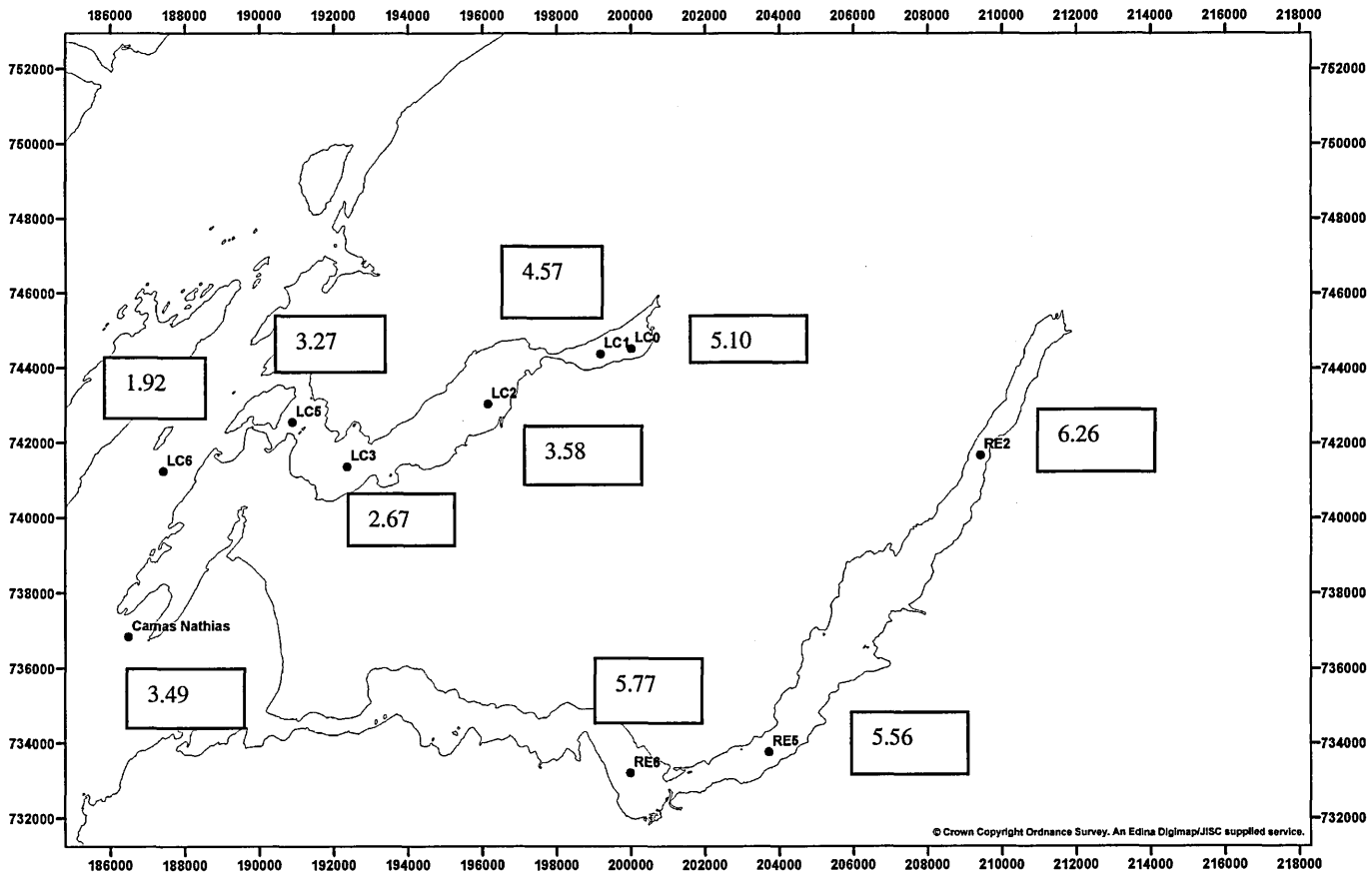
(f) Rp values due to loss on ignition.



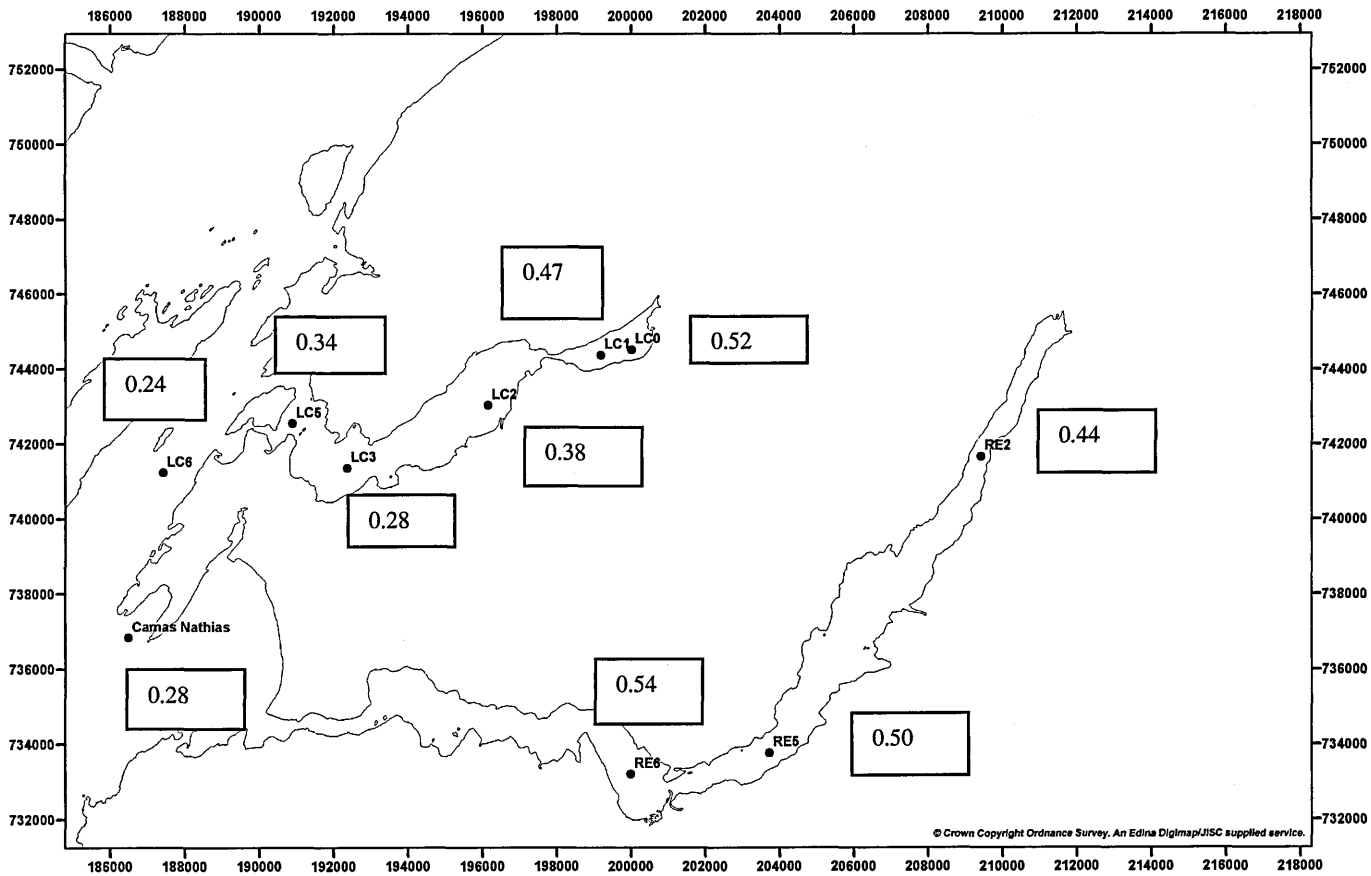
(g) $\delta^{13}C$ values (‰).



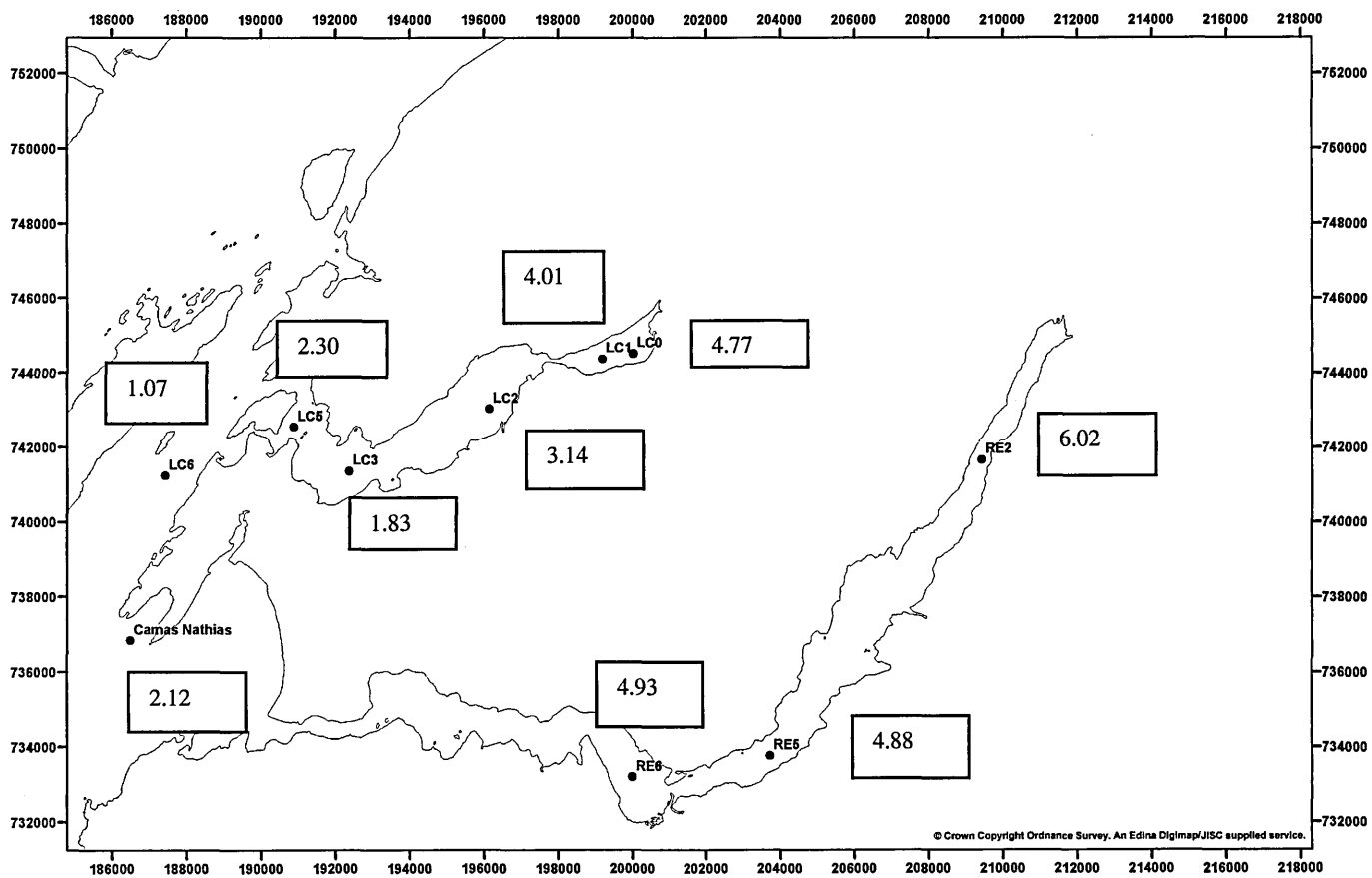
(h) Percentage TC (%).



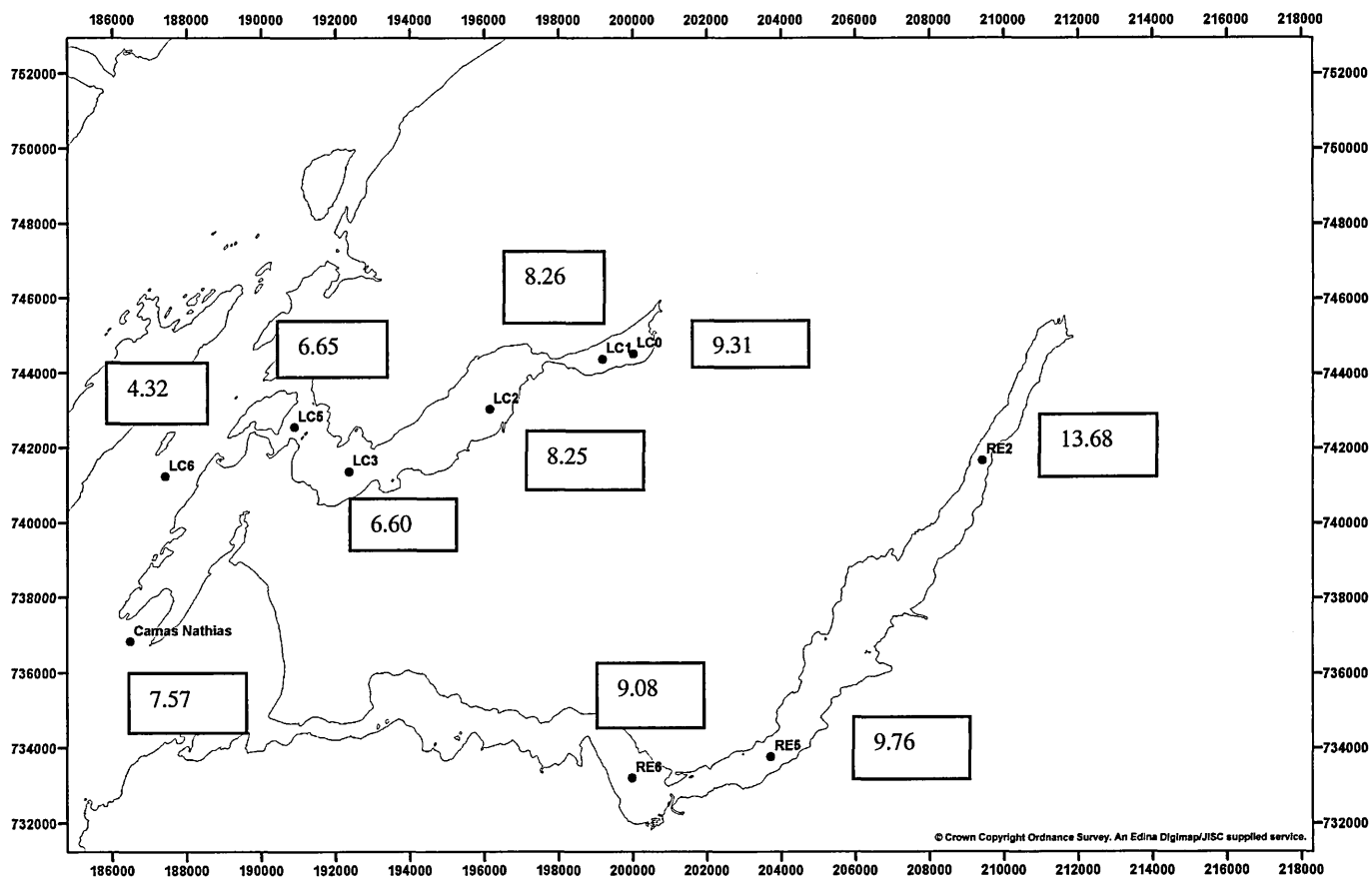
(i) Percentage TN (%).



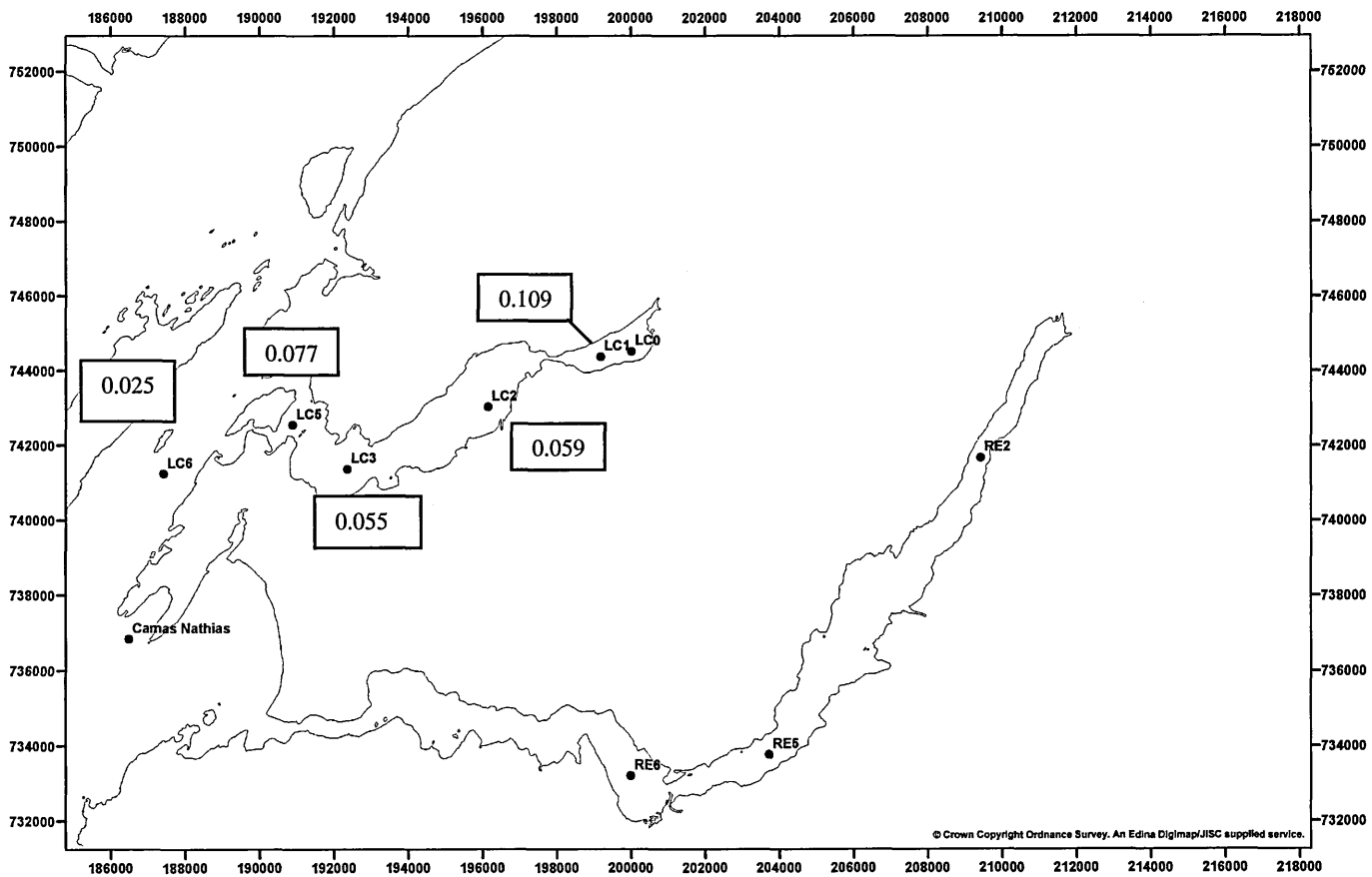
(j) Percentage TOC (%).



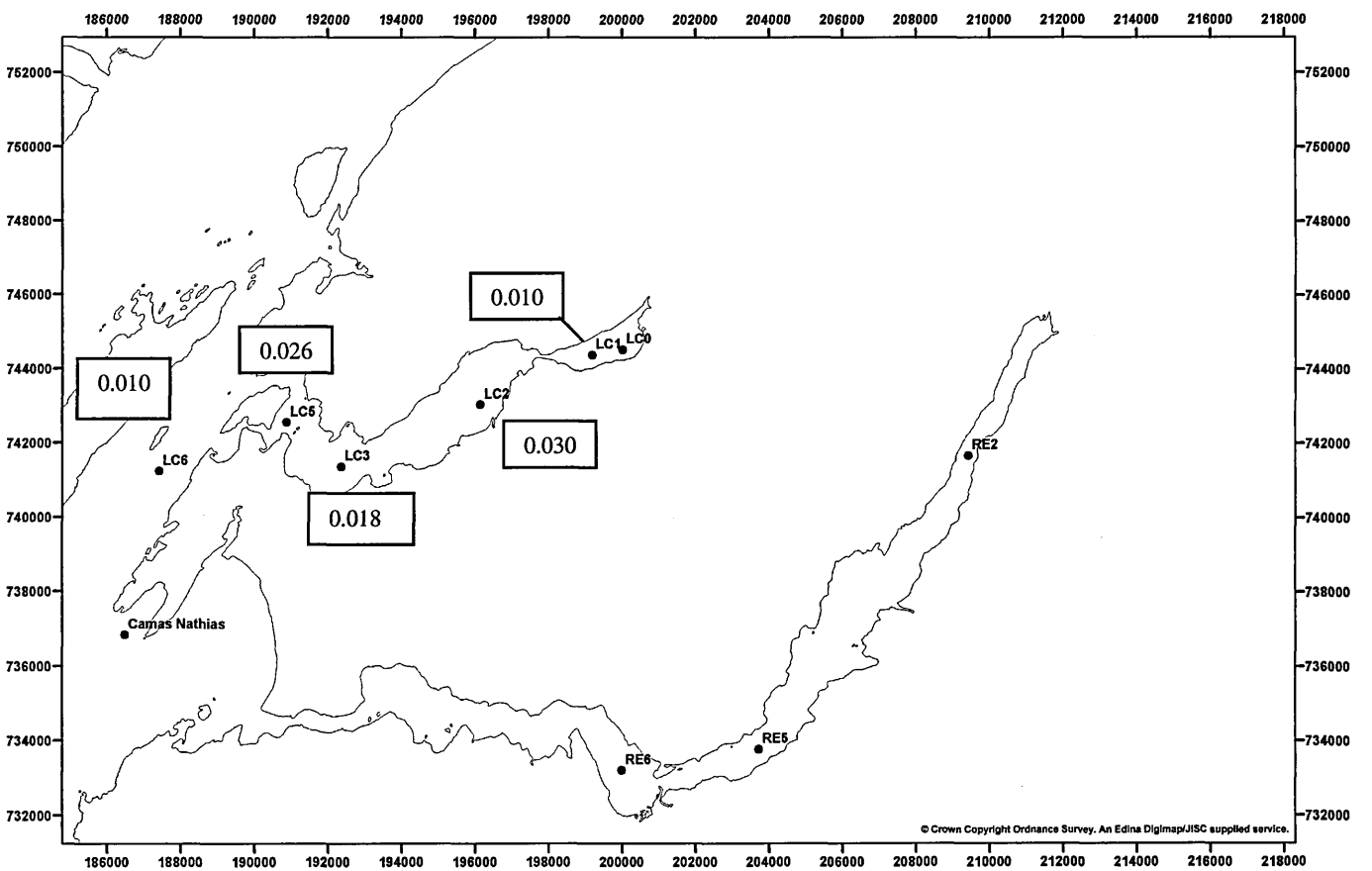
(k) The OC/N ratios.



(l) Total phosphate (µg/g).



(m) Total organic phosphate (µg/g).



(n) Total inorganic phosphate (µg/g).